CIB Forensic Science Center
Training Seminar (Taipei, Taiwan)
June 6-7, 2012

DNA Mixture Interpretation & Statistical Analysis

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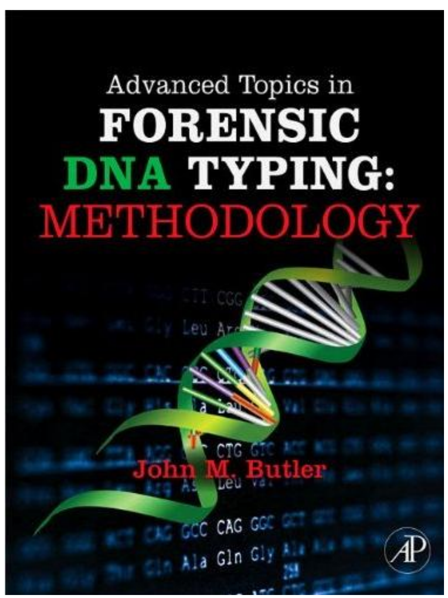
Steps Involved in Process
of Forensic DNA Typing

1) Data Interpretation
2) Statistical Interpretation

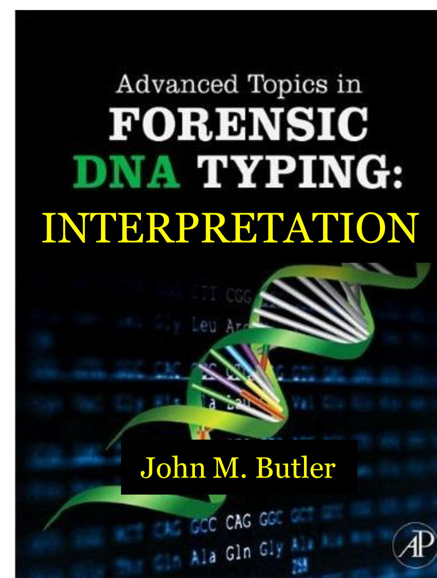
Gathering the Data | **Understanding the Data**

Collection/Storage/ Characterization → **Extraction/ Quantitation** → **Amplification/ Marker Sets** → **Separation/ Detection** → **Interpretation** → **Report**

Advanced Topics: Methodology



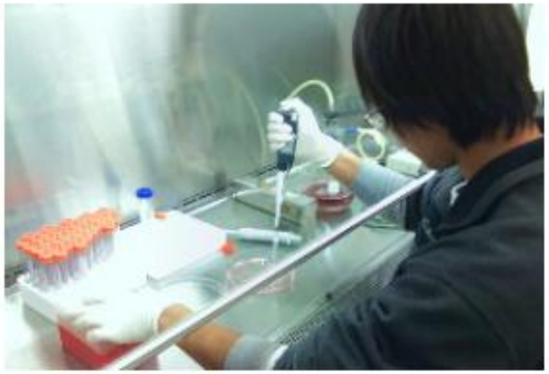
Advanced Topics: Interpretation

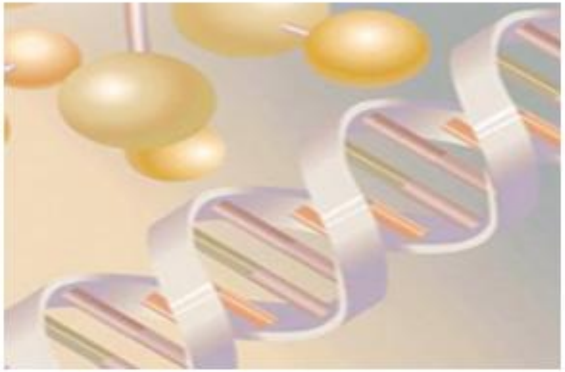


SWGDAM Website and Resources Available

http://www.swgdam.org/resources.html

- Home
- ByLaws
- Members
- Committees
- Meetings
- Publications





Additional Resources

Beginning with the development or/and revision of its next draft guidance document(s), SWGDAM will make a "Draft for Comment" or other work product available for the purpose of receiving comments from the general public. This "Draft for Comment" solicitation will be open for a minimum of 60 days, usually through SWGDAM.org. SWGDAM will make all reasonable efforts to advise the forensic DNA community of the open comment period for a proposed guidance document or standard, guideline, best practice, study, or other recommendation and/or finding via as many avenues as possible to include posting notices through discipline-specific and related professional organizations. SWGDAM strongly encourages all interested parties to regularly monitor SWGDAM.org for the posting of such draft documents as well. All public comments received by SWGDAM will forwarded to the appropriate SWGDAM Committee for review and consideration as a part of its formal business practice for the development of the guidance documents or other work product.

The following information resources have been produced and reviewed by members of the ***Mixture Committee*** *of SWGDAM and are available at*
www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Link to **http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm**

Mixture Training Materials

Reviewed by SWGDAM Mixture Committee

SWGDAM Mixture Committee Resource Page

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*The following information resources have been produced and reviewed by members of the **Mixture Committee** of the Scientific Working Group on DNA Analysis Methods (SWGDAM) -- see* http://www.swgdam.org/resources.html *for additional information.*

Mixture Training Examples

- Download "Mixture 6" PowerPoint show (56 Mb)

  - with voice-over by Bruce Heidebrecht (Maryland State Police); may work best if file is first saved to your computer

- Download "Mixture IQAS2904" PowerPoint show (35 Mb)

  - with voice-over by Bruce Heidebrecht (Maryland State Police); may work best if file is first saved to your computer

http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm

Mixture Workshop (Promega ISHI 2010)

http://www.cstl.nist.gov/biotech/strbase/mixture.htm

October 11, 2010

Regional workshops presented in FL, TX, MI, and AZ (April – June 2011)

Updated mixture workshop presented at ISHI 2011 (October 3, 2011)

Handout >200 pages

Literature list of >100 articles

13 Modules Prese

Introductio
SWGD (John)
A **olds** (Catherine)
e)
astic effects (Robin)
eak height ratios (Charlotte)
Number of contributors (John)
Mixture ratios (John)
Mixture principles (Charlotte)
Statistics (Mike)

Case Example 1 (Robin)
Case Example 2 (Charlotte)
Case Example 3 (John)

Catherine Grgicak	**Mike Coble**	**Robin Cotton**	**John Butler**	**Charlotte Word**
Boston U.	NIST	Boston U.	NIST	Consultant

NIJ Grant to Boston University funded ~150 state & local lab analysts to attend

Promega ISHI 2012 Mixture Workshop

- **John Butler,** Ph.D., NIST, Gaithersburg, MD
- **Michael Coble**, Ph.D., NIST, Gaithersburg, MD
- **Robin Cotton**, Ph.D., Boston University, Boston, MA
- **Catherine Grgicak**, Ph.D., Boston University, Boston, MA
- **Charlotte J. Word**, Ph.D., Gaithersburg, MD

This workshop is for analysts, technical reviewers and technical leaders performing and interpreting validation studies and/or interpreting and reviewing STR data, particularly more difficult mixtures. Various DNA profiles will be analyzed and interpreted using selected analytical thresholds and stochastic thresholds to demonstrate the impact of those values on the profiles amplified with low-template DNA vs. higher amounts of DNA. Different statistical approaches and conclusions suitable for the profiles will be presented.

Useful Articles on DNA Mixture Interpretation

- Buckleton, J.S. and Curran, J.M. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

- Budowle, B., *et al.* (2009) Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *J. Forensic Sci.* 54: 810-821.

- Clayton, T.M., *et al.* (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Sci. Int.* 91: 55-70.

- Gill, P., *et al.* (2006) DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101.

- Gill, P., *et al.* (2008) National recommendations of the technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes. *FSI Genetics* 2(1): 76–82.

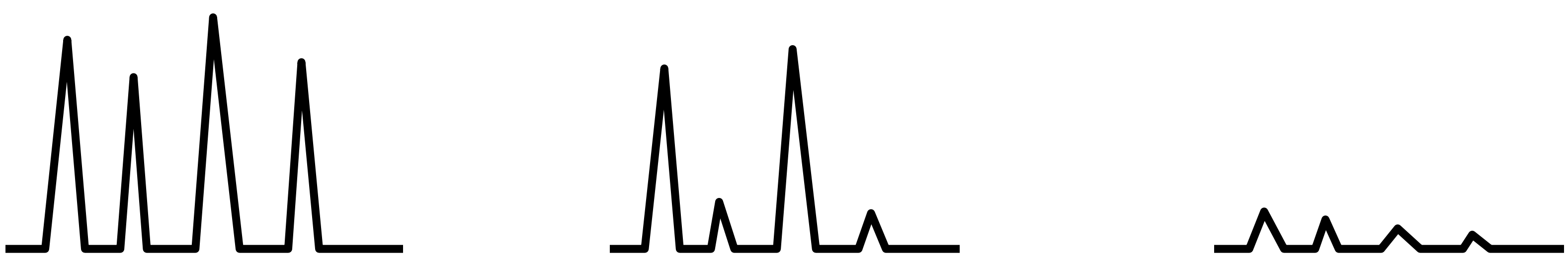
- Schneider, P.M., *et al.* (2009) The German Stain Commission: recommendations for the interpretation of mixed stains. *Int. J. Legal Med.* 123: 1-5.

German Mixture Classification Scheme

Schneider *et al*. (2009) *Int. J. Legal Med*. 123: 1-5

(German Stain Commission, 2006):

- **Type A**: no obvious major contributor, no evidence of stochastic effects
- **Type B**: clearly distinguishable major and minor contributors; consistent peak height ratios of ***approximately 4:1*** (major to minor component) for all heterozygous systems, no stochastic effects
- **Type C**: mixtures without major contributor(s), evidence for stochastic effects



Type A | **Type B** | **Type C**

"Indistinguishable" | **"Distinguishable"** | **"Uninterpretable"**

SWGDAM

Available for download from the ISFG Website:
http://www.isfg.org/Publication;Gill2006

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Forensic Science International 160 (2006) 90–101

Forensic Science International
www.elsevier.com/locate/forsciint

DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

P. Gill [a,*], C.H. Brenner [b], J.S. Buckleton [c], A. Carracedo [d], M. Krawczak [e], W.R. Mayr [f], N. Morling [g], M. Prinz [h], P.M. Schneider [i], B.S. Weir [j]

[a] *Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK*
[b] *Forensic Science Group, School of Public Health, University of California, Berkeley, CA 510-339-1911, USA*
[c] *ESR, Private Bag 92021, Auckland, New Zealand*

Our discussions have highlighted a significant need for continuing education and research into this area.

[j] *University of Washington, Department of Biostatistics, Box 357232, Seattle, WA 98195, USA*

Received 4 April 2006; accepted 10 April 2006
Available online 5 June 2006

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
2. Scientists should be trained in and use LRs
3. Methods to calculate LRs of mixtures are cited
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
7. Allele dropout to explain evidence can only be used with low signal data
8. No statistical interpretation should be performed on alleles below threshold
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

Forensic Science International
91 (1998) 55–70

Analysis and interpretation of mixed forensic stains using DNA STR profiling

T.M. Clayton[a,*], J.P. Whitaker[a], R. Sparkes[b], P. Gill[b]

[a] *Forensic Science Service, Wetherby Laboratory, Sandbeck Way, Audby Lane, Wetherby, West Yorkshire LS22 4DN, UK*
[b] *Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK*

Received 13 May 1997; received in revised form 9 October 1997; accepted 27 October 1997

Steps in the interpretation of mixtures

(Clayton *et al.* *Forensic Sci. Int.* 1998; 91:55-70)

Step #1 — Identify the Presence of a Mixture

↓

Step #2 — Designate Allele Peaks

↓

Step #3 — Identify the Number of Potential Contributors

↓

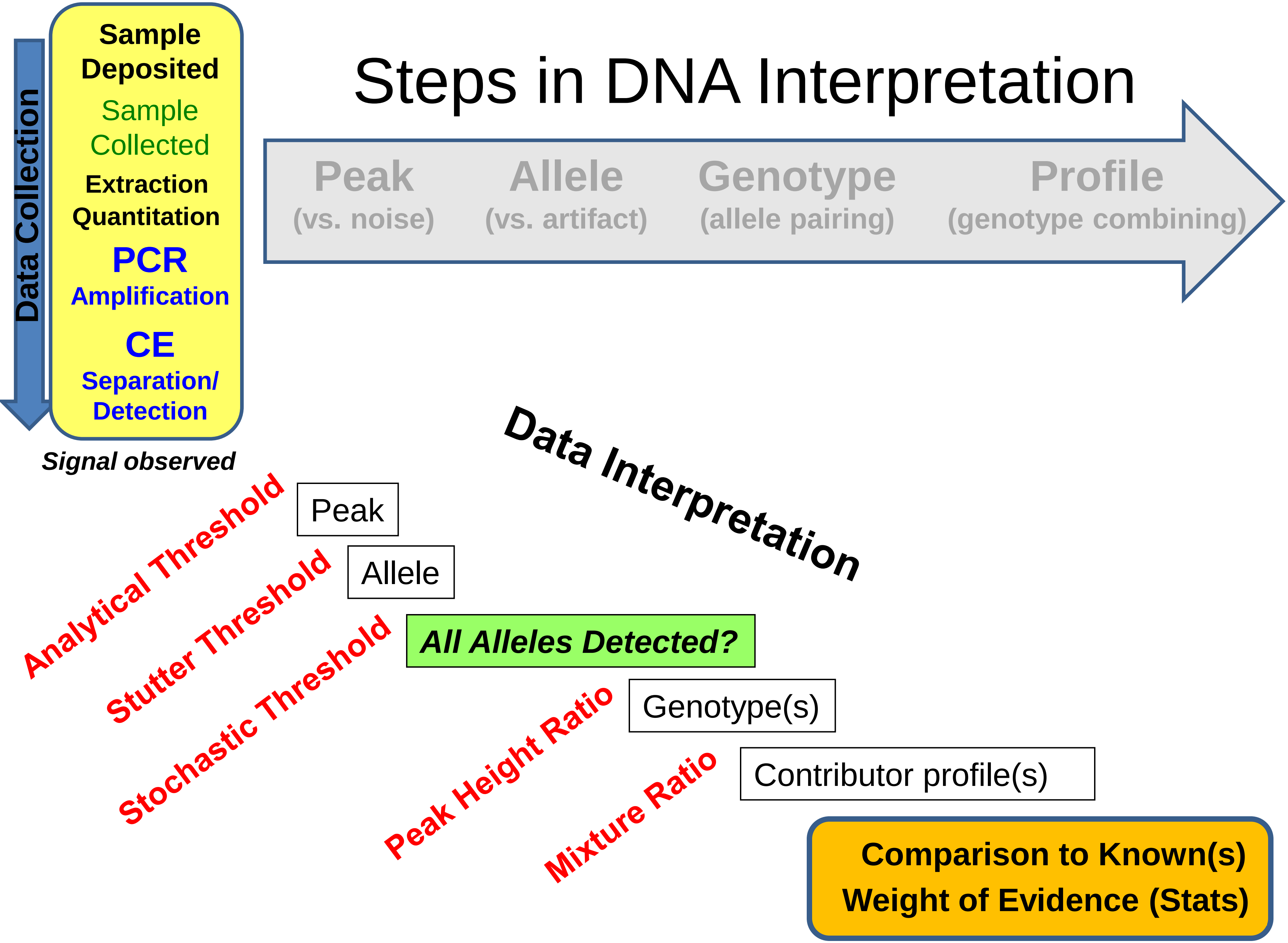
Step #4 — Estimate the Relative Ratio of the Individuals Contributing to the Mixture

↓

Step #5 — Consider All Possible Genotype Combinations

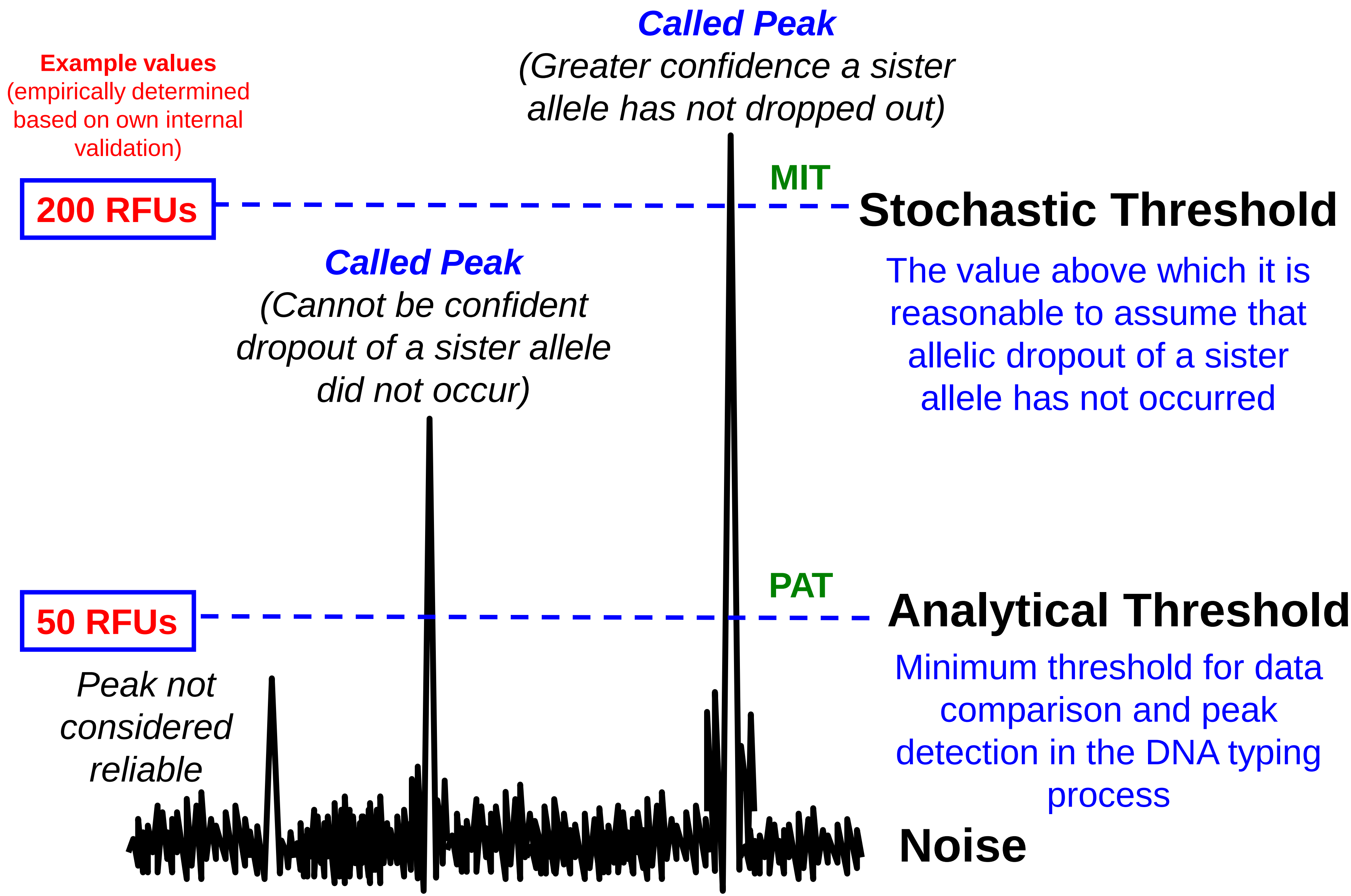
↓

Step #6 — Compare Reference Samples



Steps in DNA Interpretation
Data Collection
Sample Deposited
Sample Collected
Extraction
Quantitation
PCR
Amplification
CE
Separation/ Detection
Signal observed
Peak
(vs. noise)
Allele
(vs. artifact)
Genotype
(allele pairing)
Profile
(genotype combining)
Data Interpretation
Analytical Threshold
Stutter Threshold
Stochastic Threshold
Peak Height Ratio
Mixture Ratio
Peak
Allele
All Alleles Detected?
Genotype(s)
Contributor profile(s)
Comparison to Known(s)
Weight of Evidence (Stats)

Overview of Two Thresholds



Butler, J.M. (2010) *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press: San Diego.

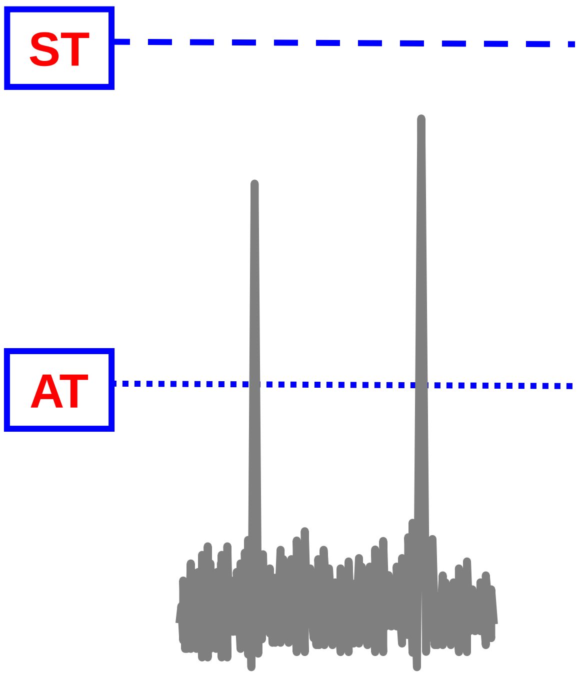
Coupling of Statistics and Interpretation

- **The CPE/CPI approach** for reporting an inclusionary statistic **requires that all alleles be observed** in the evidence sample

- If allele drop-out is suspected at a locus, then any allele is possible and the probability of inclusion goes to 100% -- in other words, the locus is effectively dropped from consideration

- If alleles are seen below the established stochastic threshold, then the locus is typically eliminated (“INC” – declared inconclusive) in many current lab SOPs

Can This Locus Be Used for Statistical Calculations?



It depends on your assumption as to the number of contributors!

If you assume <u>a single-source sample</u>, then you can assume that the detection of two alleles fully represents the heterozygous genotype present at this locus.

If you assume (from examining other loci in the profile as a whole) that the sample is a mixture of two or more contributors, then there may be allele drop-out and all alleles may not be fully represented.

Limitations of Stochastic Thresholds

- The possibility of allele sharing with a complex mixture containing many contributors may make a stochastic threshold meaningless

- “Enhanced interrogation techniques” to increase sensitivity (e.g., increased PCR cycles) may yield false homozygotes with >1000 RFU

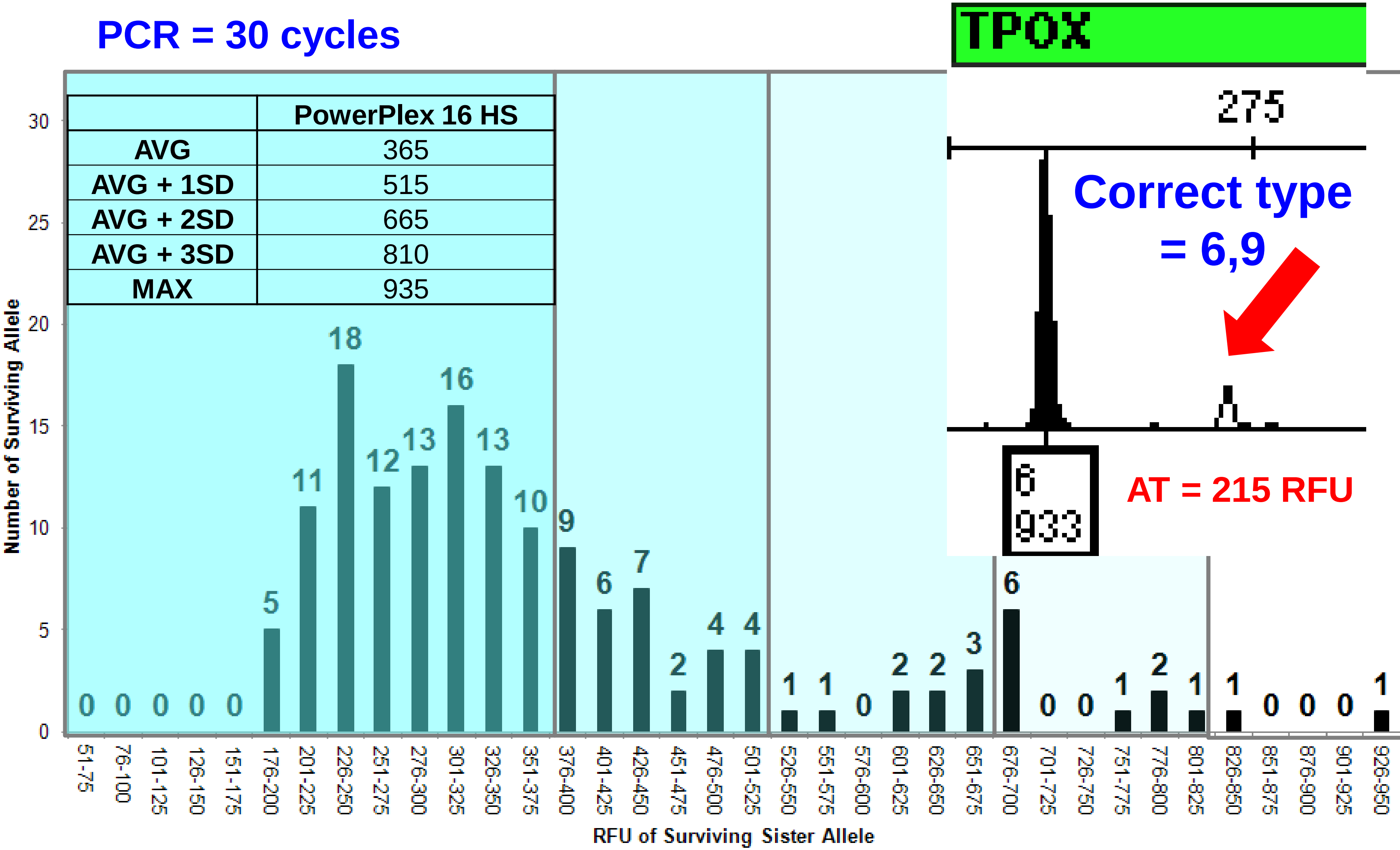
- **New turbo-charged kits with higher sensitivity will need to be carefully evaluated to avoid allele drop-out and false homozygotes**

PowerPlex 16 HS Stochastic Threshold

(ABI 3500 Data – **see Poster #42**)

PCR = 30 cycles

	PowerPlex 16 HS
AVG	365
AVG + 1SD	515
AVG + 2SD	665
AVG + 3SD	810
MAX	935



Stochastic Threshold Summary

- A stochastic threshold (ST) may be established for a specific set of conditions to reflect possibility of allele drop-out, which is essential for a CPE/CPI stats approach
- ST should be re-examined with different conditions (e.g., higher injection, sample desalting, increase in PCR cycles)
- ST will be dependent on the analytical threshold set with a method and impacts the lowest expected peak height ratio
- Assumptions of the number of contributors is key to correct application of ST

Stats Required for Inclusions

SWGDAM Interpretation Guideline 4.1:

"**The laboratory must perform statistical analysis in support of any inclusion** that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis."

Buckleton & Curran (2008): "There is a considerable aura to DNA evidence. Because of this aura **it is vital that weak evidence is correctly represented as weak or not presented at all**."

Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

DAB Recommendations on Statistics

February 23, 2000

Forensic Sci. Comm. 2(3); available on-line at http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm

"The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated"

- Probability of exclusion (PE)
  - Devlin, B. (1993) Forensic inference from genetic markers. *Statistical Methods in Medical Research* 2: 241–262.
- Likelihood ratios (LR)
  - Evett, I. W. and Weir, B. S. (1998) *Interpreting DNA Evidence*. Sinauer, Sunderland, Massachusetts.

CPE/CPI (RMNE) Limitations

- A CPE/CPI approach assumes that all alleles are present (i.e., cannot handle allele drop-out)

- Thus, statistical analysis of low-level DNA CANNOT be correctly performed with a CPE/CPI approach because some alleles may be missing

- Charles Brenner in his AAFS 2011 talk addressed this issue

- Research is on-going to develop allele drop-out models and software to enable appropriate calculations

Notes from Charles Brenner's AAFS 2011 talk

The Mythical "Exclusion" Method for Analyzing DNA Mixtures – Does it Make Any Sense at All?

1. The claim that is requires **no assumption about number of contributors** is mostly wrong.
2. The supposed **ease of understanding** by judge or jury is really an illusion.
3. **Ease of use** is claimed to be an advantage particularly for complicated mixture profiles, those with many peaks of varying heights. The truth is the exact opposite. **The exclusion method is completely invalid for complicated mixtures.**
4. The exclusion method is only **conservative** for guilty suspects.

- "Certainly no one has laid out an explicit and rigorous chain of reasoning from first principles to support the exclusion method. It is at best guesswork."

Brenner, C.H. (2011). The mythical "exclusion" method for analyzing DNA mixtures – does it make any sense at all? *Proceedings of the American Academy of Forensic Sciences*, Feb 2011, Volume 17, p. 79

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

- Discussion about exclusion probabilities in Paternity cases.

Two types:

(1) Conditional Exclusion Probability - excluding a random man as a possible father, given the mother-child genotypes for a particular case.

(2) Average Exclusion Probability – excluding a random man as a possible father, given a randomly chosen mother-child pair.

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

“The theoretical concept of exclusion probabilities, however, makes no sense within the framework of normal mixture models.”

“The interpretation of conditional exclusion probability is obvious, which accounts for its value in the legal arena. Unlike [LR], however, it is not fully efficient.”

Curran and Buckleton (2010)

JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, September 2010, Vol. 55, No. 5
doi: 10.1111/j.1556-4029.2010.01446.x
Available online at: interscience.wiley.com

PAPER

CRIMINALISTICS; GENERAL

James M. Curran,[1] M.Sc.(Hons.), Ph.D. and John Buckleton,[2] Ph.D.

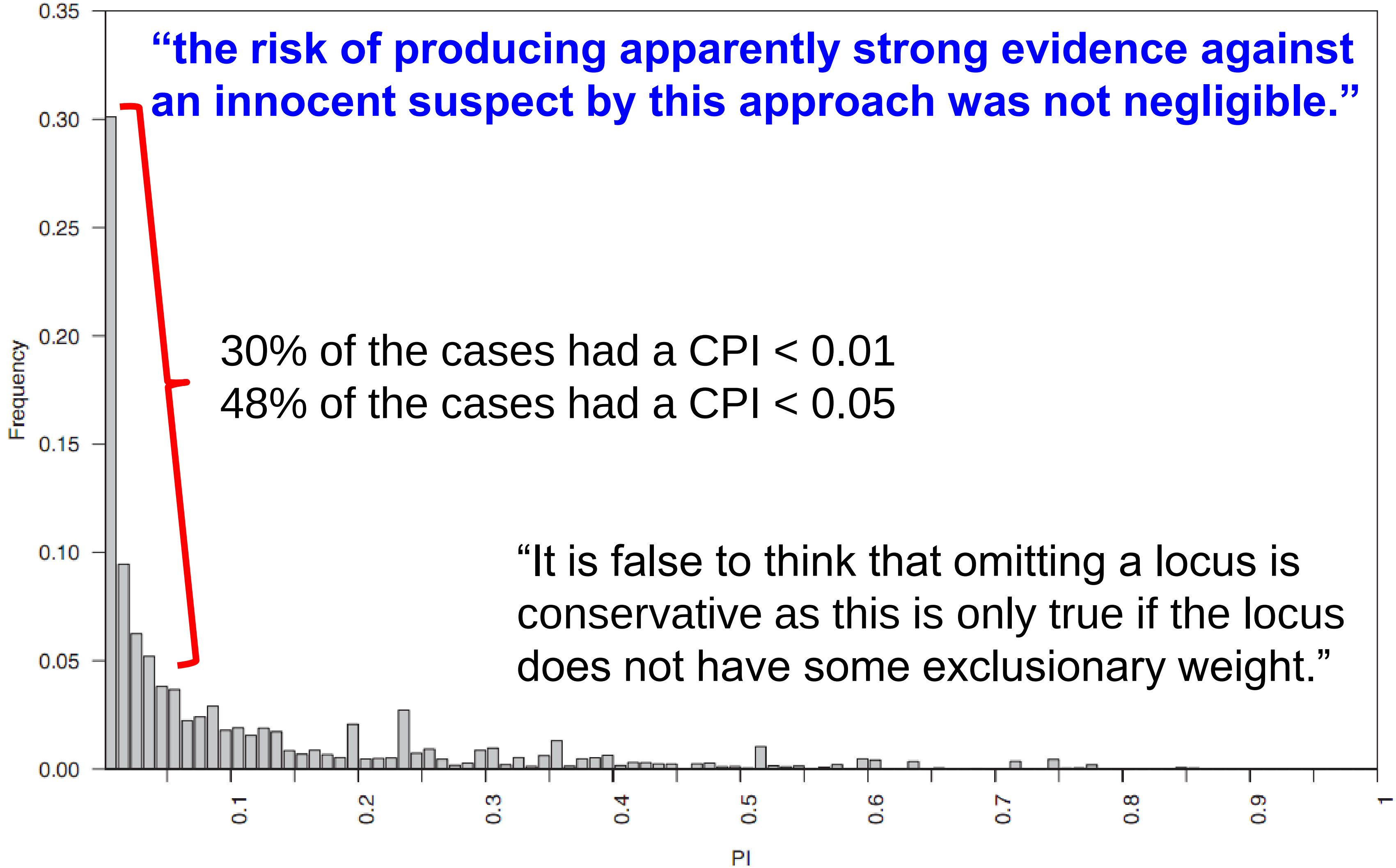
Inclusion Probabilities and Dropout

Created 1000 Two-person Mixtures (Budowle *et al.*1999 AfAm freq.).

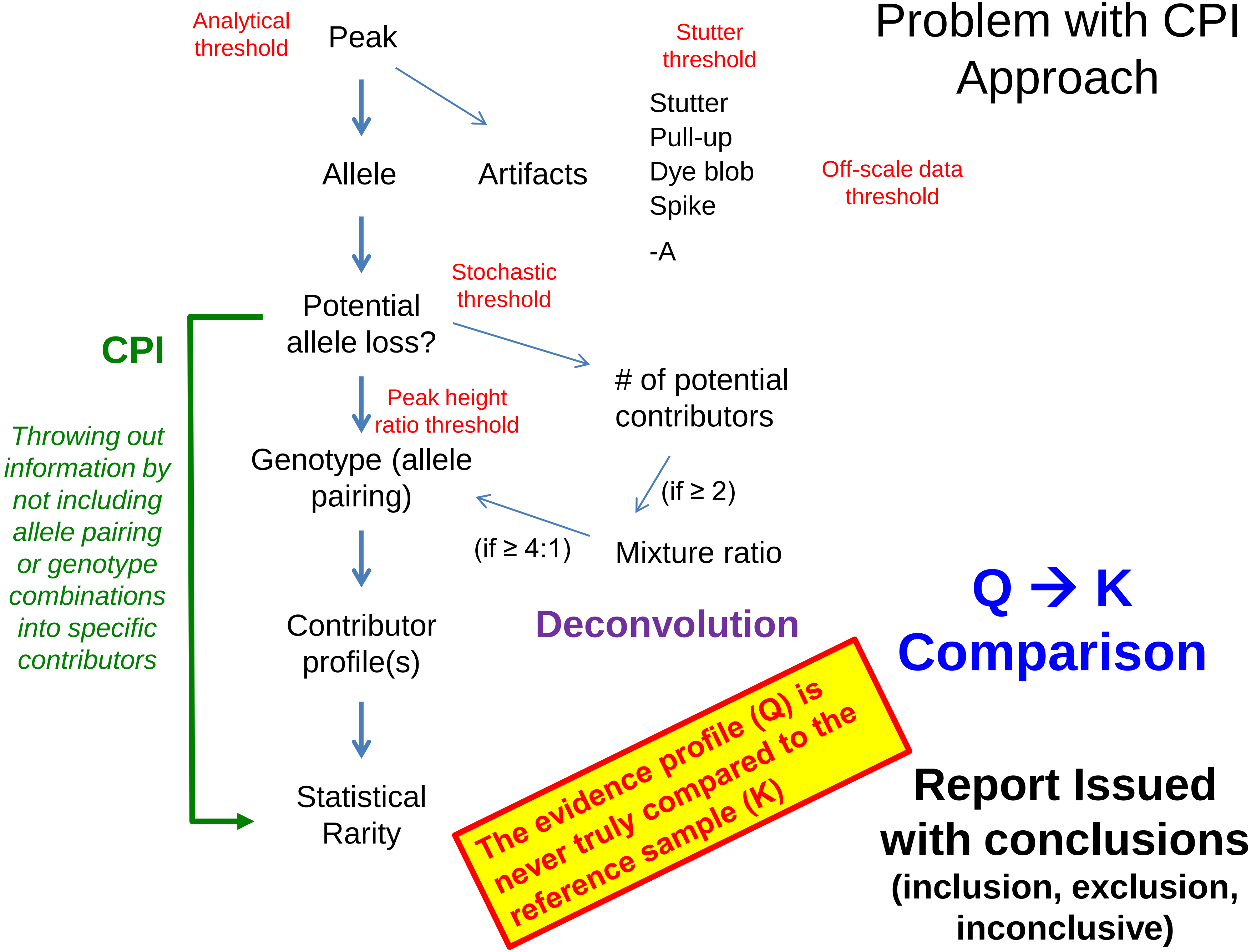
Created 10,000 “third person” genotypes.

Compared “third person” to mixture data, calculated PI for included loci, ignored discordant alleles.

Curran and Buckleton (2010)



Problem with CPI Approach



Impact of Dropping Loci

- The less data available for comparison purposes, the greater the chance of falsely including someone who is truly innocent

- Are you then being “conservative” (i.e., erring in favor of the defendant)?

Likelihood Ratio (LR)

- Provides ability to express and evaluate both the prosecution hypothesis, H_p (the suspect is the perpetrator) and the defense hypothesis, H_d (an unknown individual with a matching profile is the perpetrator)

$$LR = \frac{H_p}{H_d}$$

- **The numerator, H_p, is usually 1** – since in theory the prosecution would only prosecute the suspect if they are 100% certain he/she is the perpetrator

- The denominator, $\mathbf{H_d}$, is typically the profile frequency in a particular population (based on individual allele frequencies and assuming HWE) – i.e., **the random match probability**

Some Important Points

- Inclusionary statements (including “cannot exclude”) need statistical support to reflect the relevant weight-of-evidence
- Stochastic thresholds are necessary if using CPI statistics to help identify possible allele dropout
- CPI is only conservative for guilty suspects as this approach does a poor job of excluding the innocent
- Uncertainty exists in scientific measurements – this fact needs to be conveyed with the statistical results
- An increasing number of poor samples are being submitted to labs – labs may benefit from developing a complexity threshold

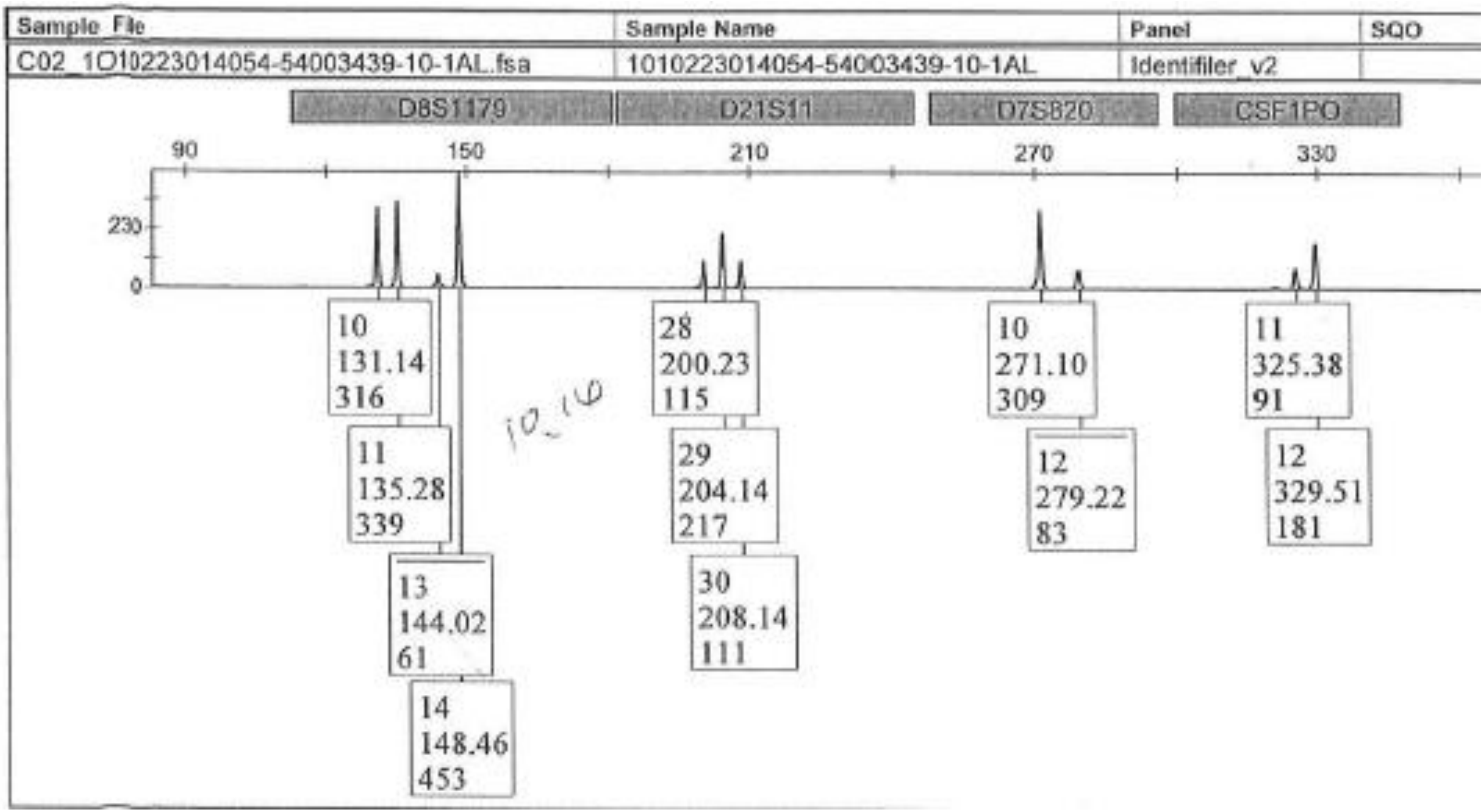
Some Mixture Examples Were Provided

- **Case 1**
  - Evidence (sexual assault victim's underwear bra)
  - Victim
  - Suspect

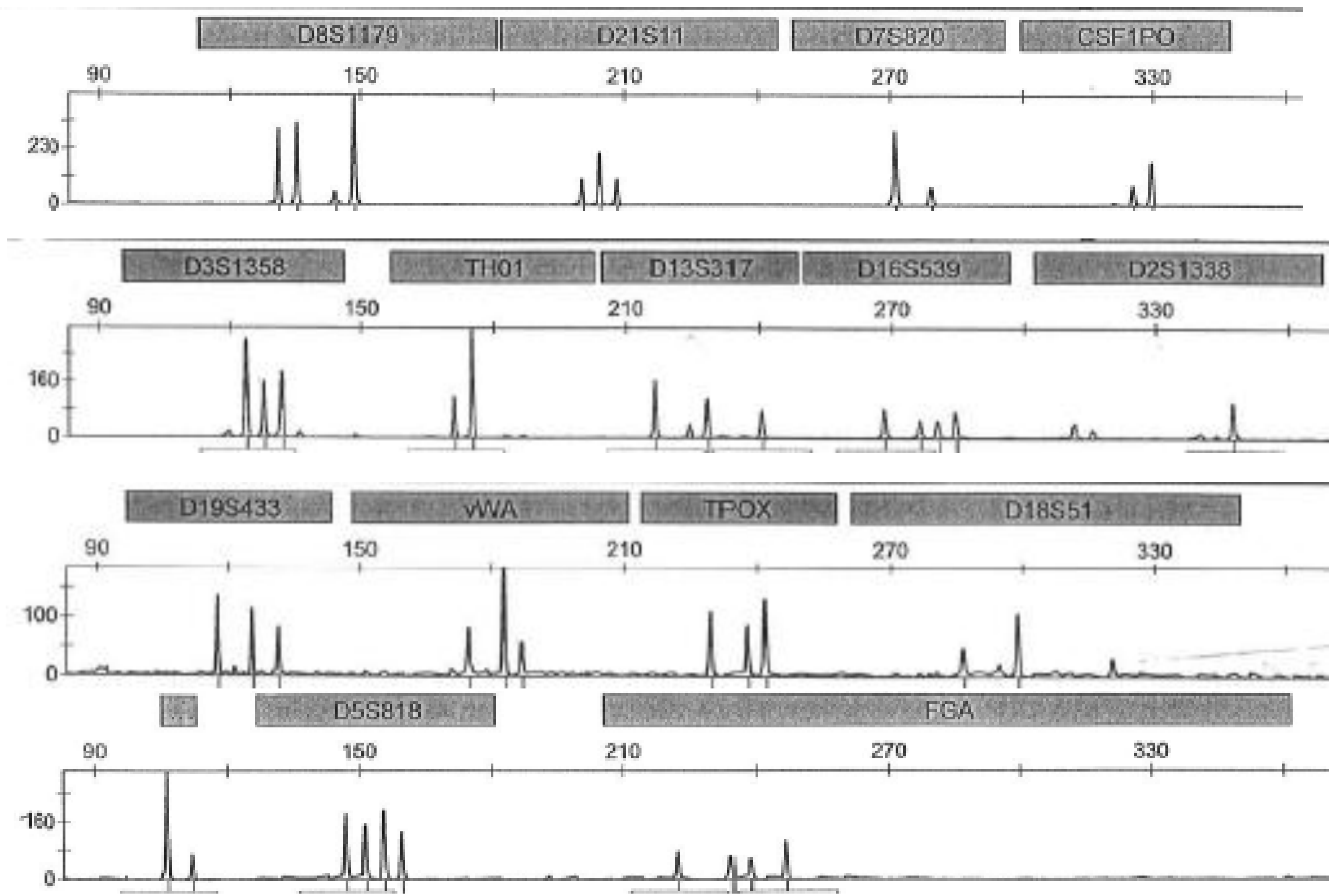
- **Case 2**
  - Evidence (sexual assault victim's panties)

- **Case 3**
  - Evidence (burglary cigarette)

Case 1 sexual assault
victim's underwear (bra)



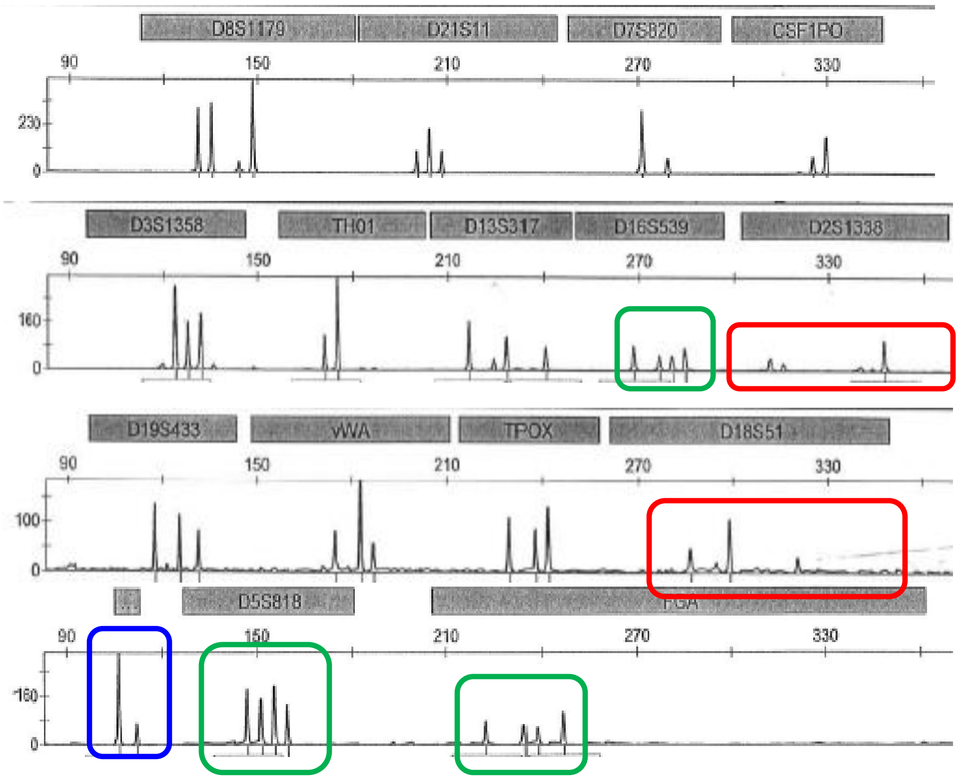
Taiwan Case 1 Evidence: Full Profile
(Identifiler)



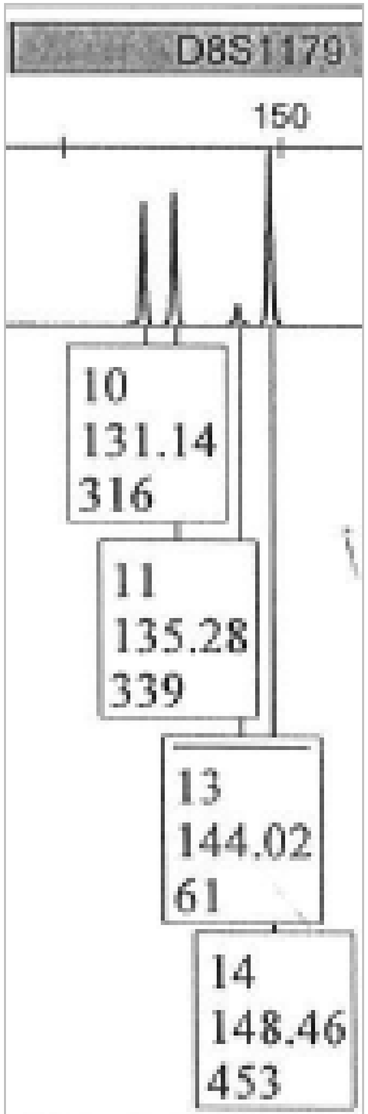
Observations from this Evidence Profile

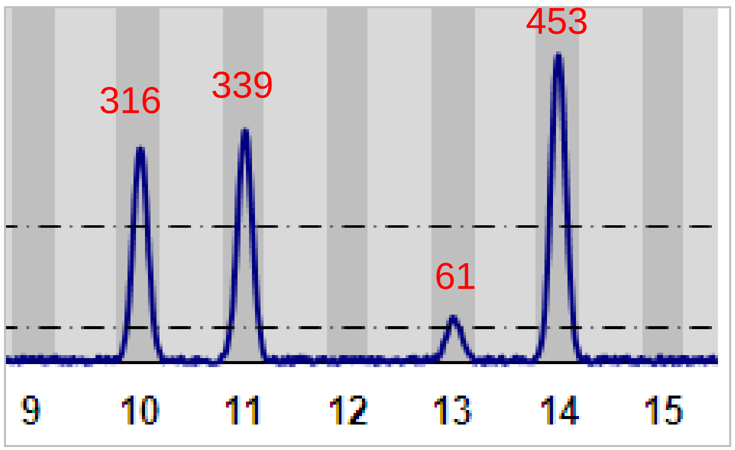
- **The sample is a mixture** since there are >2 peaks at multiple loci (at least 2 contributors)
- **Two contributors is a reasonable assumption** since there are no more than four alleles at a single locus
- **Male and female DNA are present** based on amelogenin X/Y ratio
- **A major contributor is not easily discernible** so component deconvolution is not an option
- Results at 4-allele loci (D5S818, FGA, and D16S539) suggest **≈1:1 mixture ratio**
- **Overall RFU signals are low** especially for larger loci D2S1338 and D18S51 **so allele drop-out is a possibility**

Identifiler profile (Taiwan case 1)



Case 1 Evidence: D8S1179





Is “13” a stutter of “14”?
(61/453) = 13.5%

<u>Sum of peak heights for locus</u>

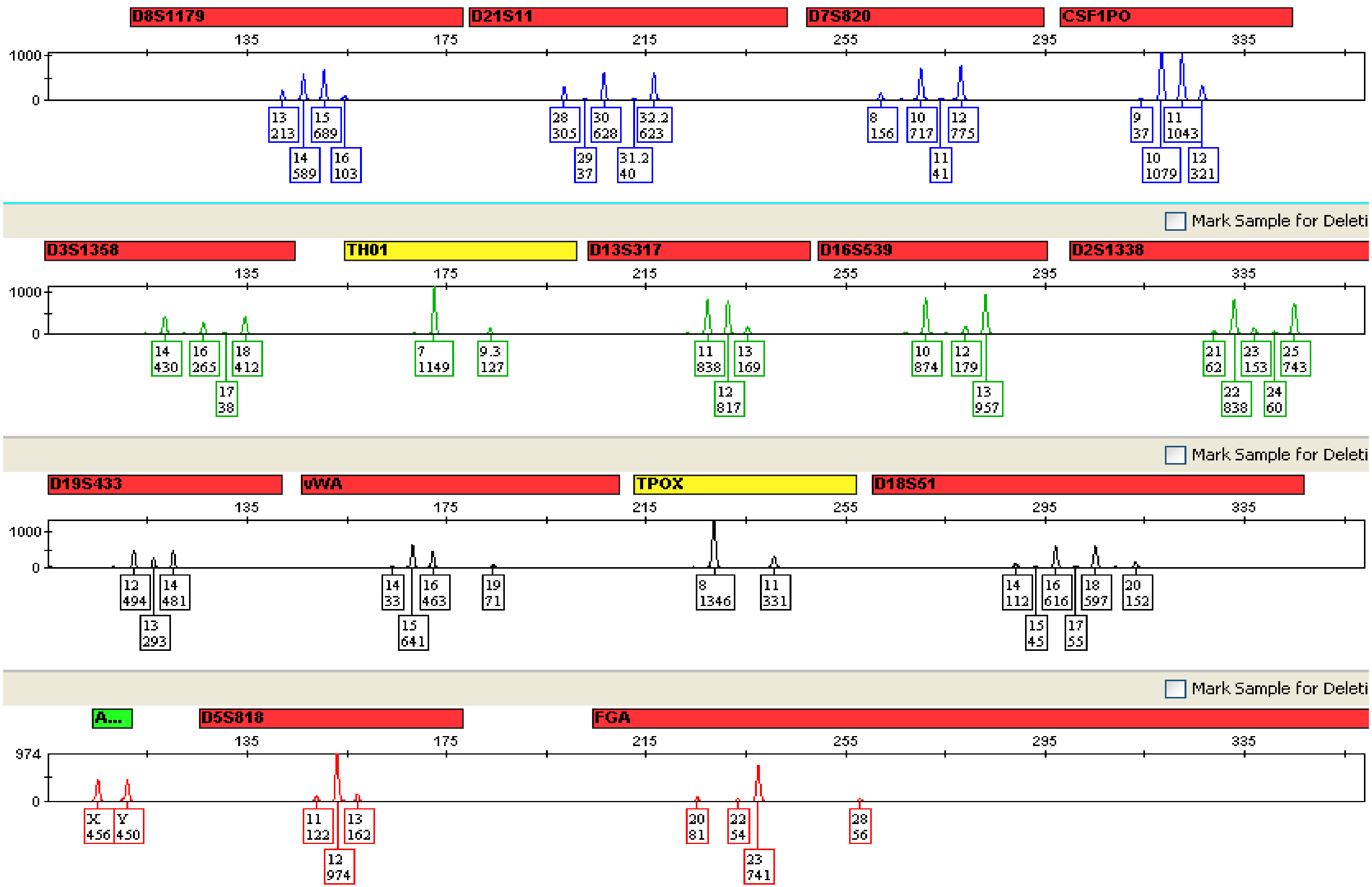
316+339+453 = 1108

316+339+61+453 = 1169

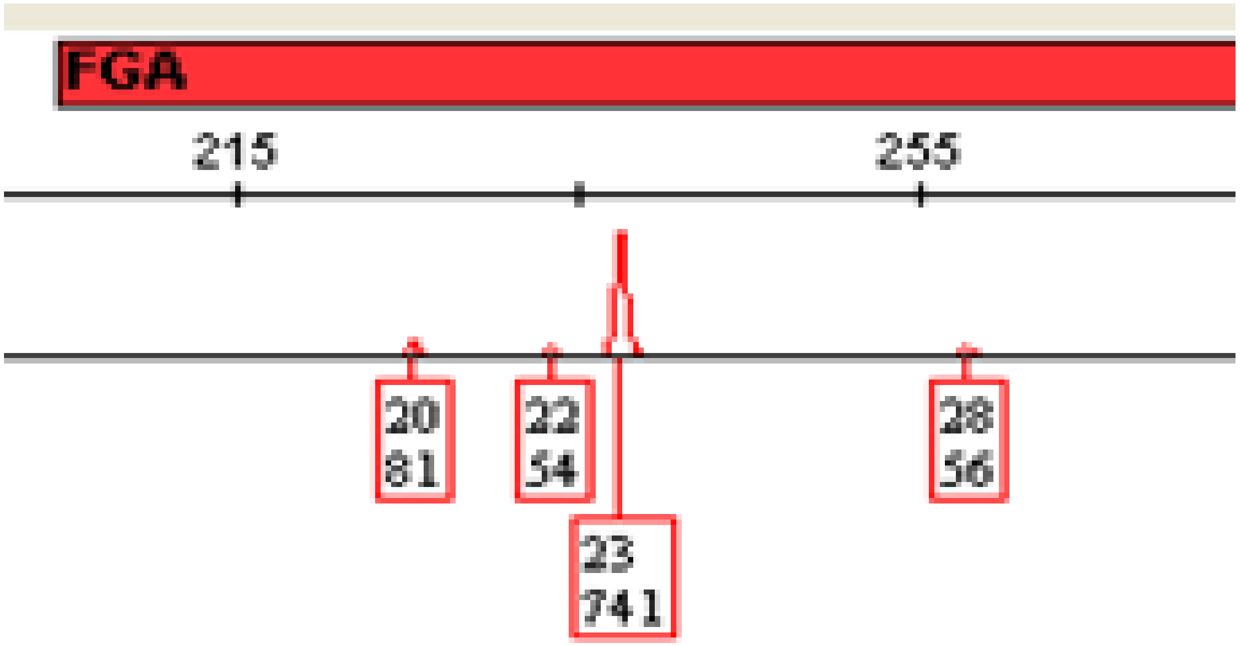
Contributor 1 Possible Genotype	**Contributor 2** Possible Genotype	**Peak Height Ratios (PHR)**	**Mixture Ratio (M_x)**
10,11	14,14	(316/339) = 93% N/A for homozygote	(316+339)/1108 = 0.59 (1.7:1)
10,10	11,14	N/A for homozygote (339/453) = 75%	
11,11	10,14	N/A for homozygote (316/453) = 70%	
10,14	11,14	(316/453) = 70% (339/453) = 75%	

SLIDES NOT COMPLETED YET on PROVIDED MIXTURE EXAMPLES

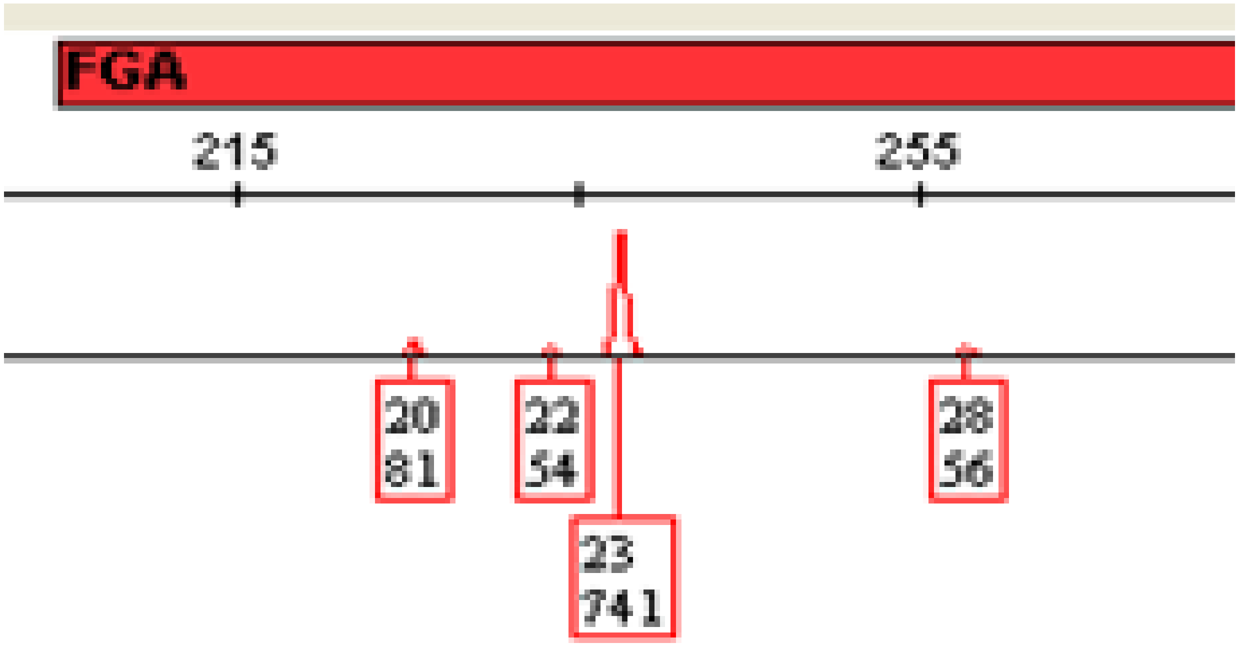
Profile 1 (stutter filter off)



Analytical Threshold (Peaks vs. Noise)

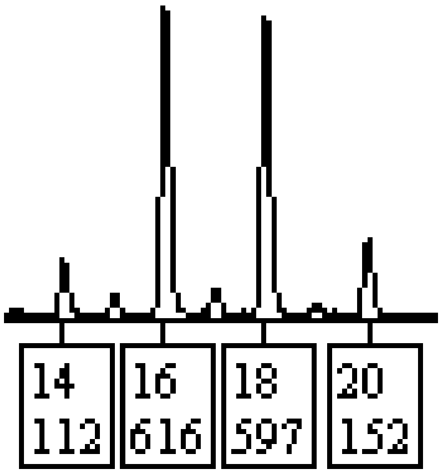


Stutter Threshold (Alelles vs. Artifacts)



Assumptions based upon # of contributors

Determination of Genotypes (PHR)



D18S51

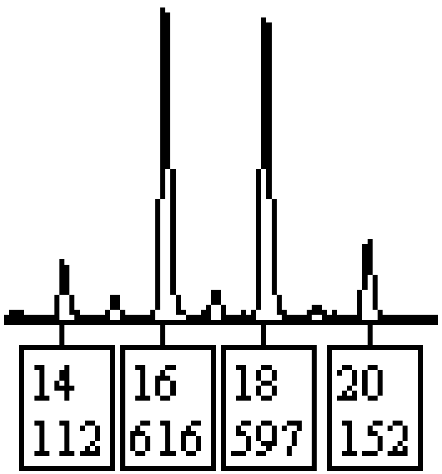
Possible Combinations

~~14, 16 and 18, 20~~
~~(18%) (25%)~~

~~14, 18 and 16, 20~~
~~(19%) (25%)~~

14, 20 and 16, 18
(74%) (97%)

Determination of Mixture Ratio



Major: 16,18
Minor: 14,20

D18S51

<u>Total of all peak heights</u>
= 112 + 616 + 597 + 152
= 1477 RFUs

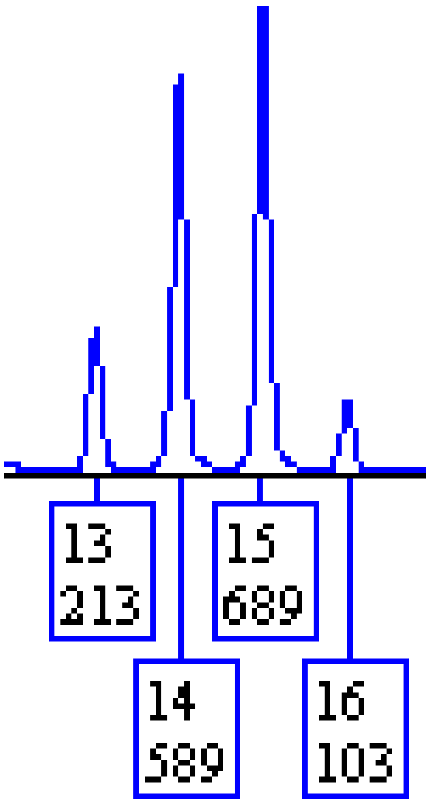
<u>Minor component</u>:
("14"+"20")/total = (112+152)/1477 = **0.179**

<u>Major component</u>:
("16"+"18")/ total = (616+597)/1477 = **0.821**

≈ 4.6 : 1

<u>Four Peaks **(4 allele loci)**</u>
heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

Determination of Genotypes (PHR)



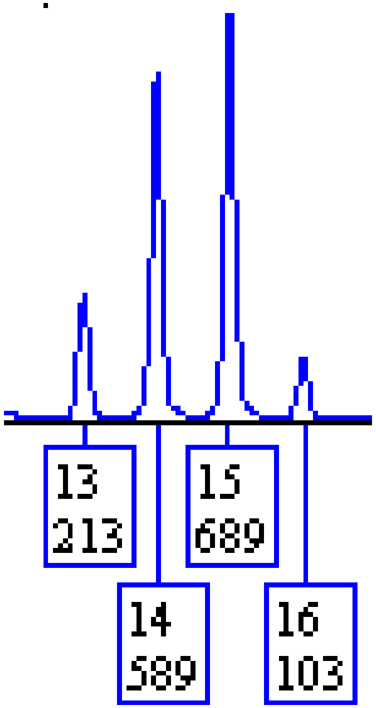
D8S1179

Possible Combinations

13, 14 (36%)	and	15, 16 (15%)
13, 15 (31%)	and	14, 16 (17%)
13, 16 (48%)	and	14, 15 (85%)

Includes “stutter”
from the 14 allele

Determination of Mixture Ratio



Total of all peak heights

= 213 + 589 + 689 + 103

= 1594 RFUs

Minor component:

("13"+"16")/total = (213+103)/1594 = **0.198**

Major component:

("14"+"15")/ total = (589+689)/1594 = **0.802**

≈ 4 : 1

Major: 14,15

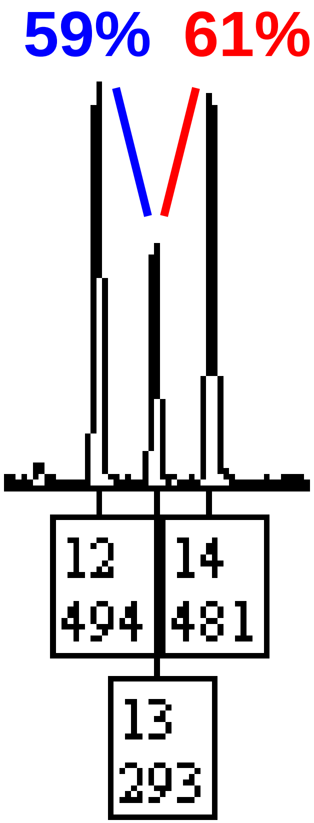
Minor: 13,16

D8S1179

Four Peaks **(4 allele loci)**

heterozygote + heterozygote, no overlapping alleles (genotypes are unique)

Application of the Mixture Ratio



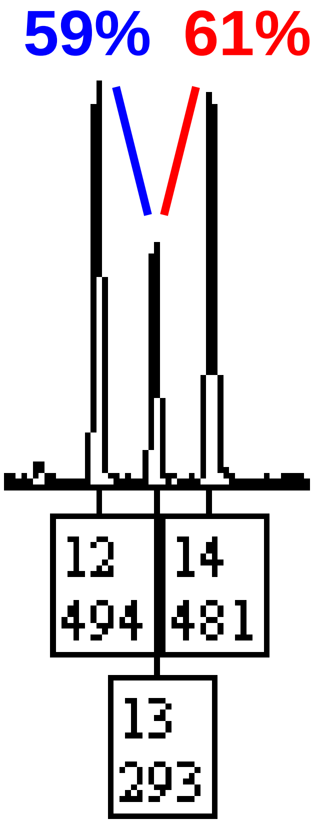
D19S433

Using peak height ratio, all genotypes possible:

12,12	**12,13**
13,13	**12,14**
14,14	**13,14**

Is there a major:minor here?

Application of the Mixture Ratio



All possible genotype combinations:

12,12 + 13,14	**1:1.6**
13,13 + 12,14	**1:3.3**
14,14 + 12,13	**1:1.6**
12,13 + 12,14	**1:1.4**
~~**12,13 + 13,14**~~	~~**1:1**~~
12,14 + 13,14	**1:1.4**

Using MIXTURE RATIO calculations, can eliminate genotype pairs

Statistical Approaches with Mixtures

See Ladd *et al.* (2001) *Croat Med J.* 42:244-246

"Exclusionary" Approach

Random Man Not Excluded (RMNE)

Combined Prob. of Inclusion (CPI)

Combined Prob. of Exclusion (CPE)

"Inferred Genotype" Approach

Random Match Probability (RMP)

Likelihood Ratio (LR)

Forensic Science International: Genetics 2 (2008) 343–348

A discussion of the merits of random man not excluded and likelihood ratios

John Buckleton [a,*], James Curran [b]

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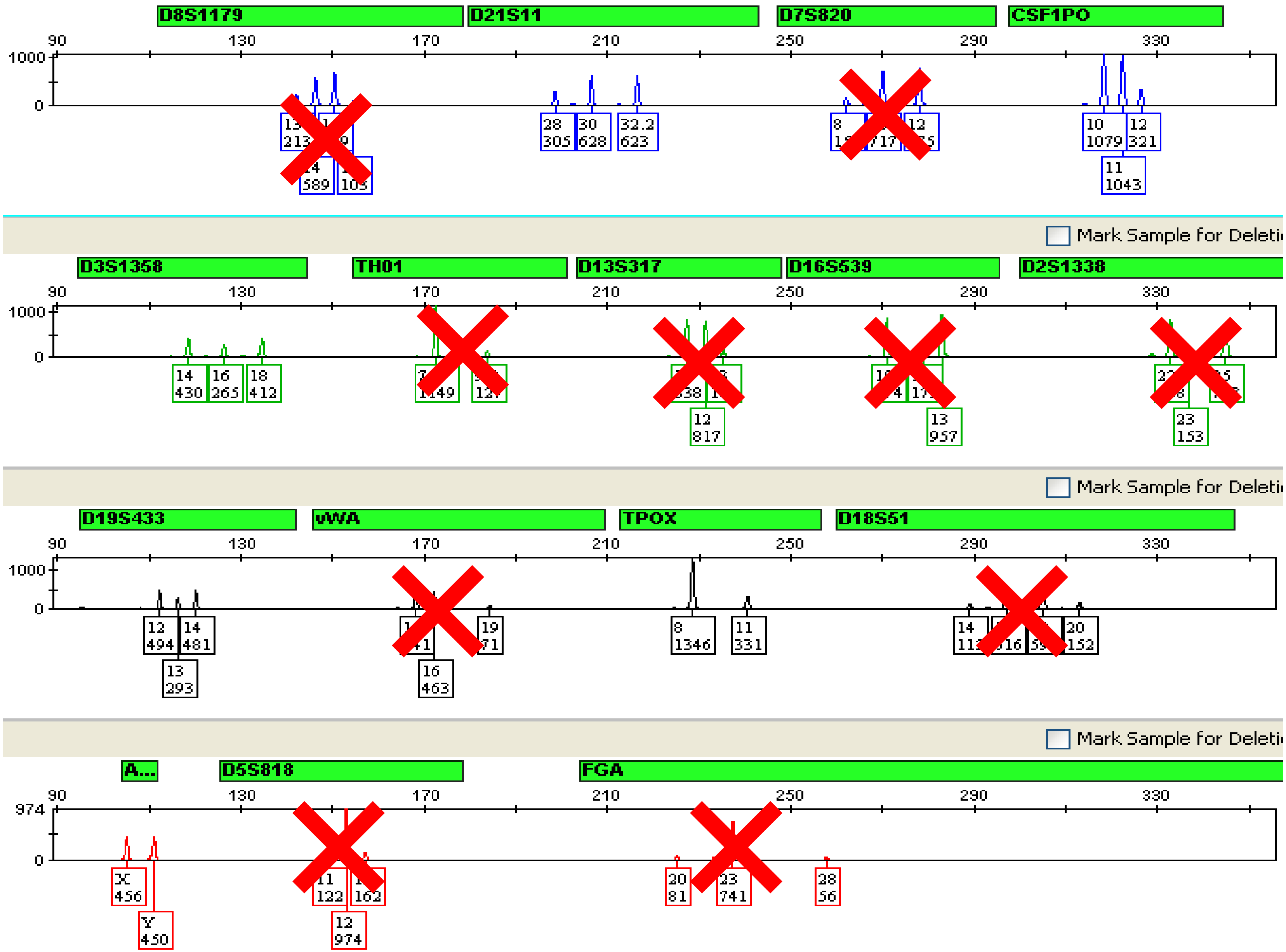
Received 15 January 2008; received in revised form 29 April 2008; accepted 1 May 2008

We conclude that the two matters that appear to have real force are:
(1) LRs are more difficult to present in court and
(2) the RMNE statistic wastes information that should be utilised.

If CPI/CPE Stats are Used

Since exclusionary statistics cannot adjust for the possibility of dropout, and does not take the number of contributors into account, any loci where alleles are below stochastic levels cannot be used in the CPI statistic.

If CPI/CPE Stats are Used



If CPI/CPE Stats are Used

Can use	Cannot use	
D21	D8	D2
CSF	D7	vWA
D3	TH01	D18
D19	D13	D5
TPOX	D16	FGA

If CPI/CPE Stats are Used

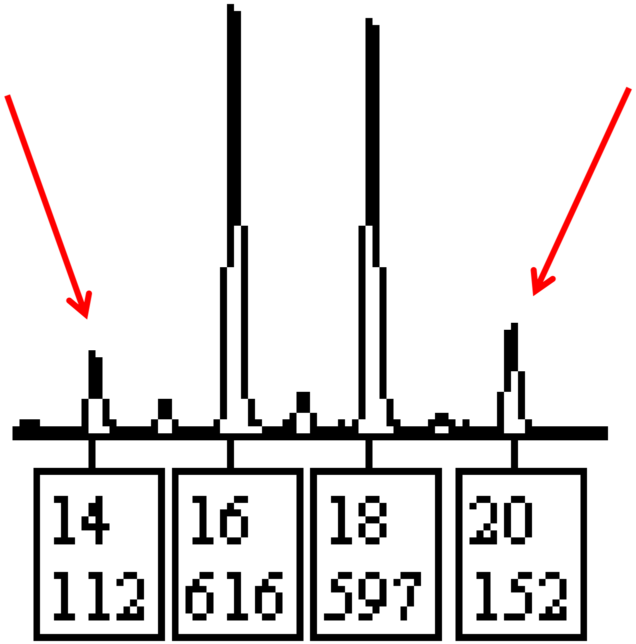
- CPI statistics using FBI Caucasian Frequencies

- 1 in 71 Caucasians included
- 98.59% Caucasians excluded

If RMP/LR Stats are Used

- Since there is an assumption to the number of contributors, it is possible to use data that falls below the ST.

RMP - D18S51



If Assume 2 Contributors….

Major	Minor
16,18	14,20

$$RMP_{minor} = 2pq$$

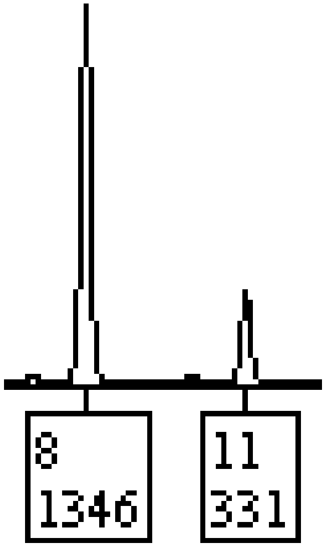
$$= 2 \times f(14) \times f(20)$$

$$= 2 \times (0.1735) \times (0.0255)$$

$$= 0.00884 \quad \text{or 1 in 113}$$

(LR = 113)

RMP - TPOX



If Assume 2 Contributors….

<u>Major</u>	<u>Minor</u>
8,8	11,8 *OR* 11,11

RMP = 8,11 + 11,11

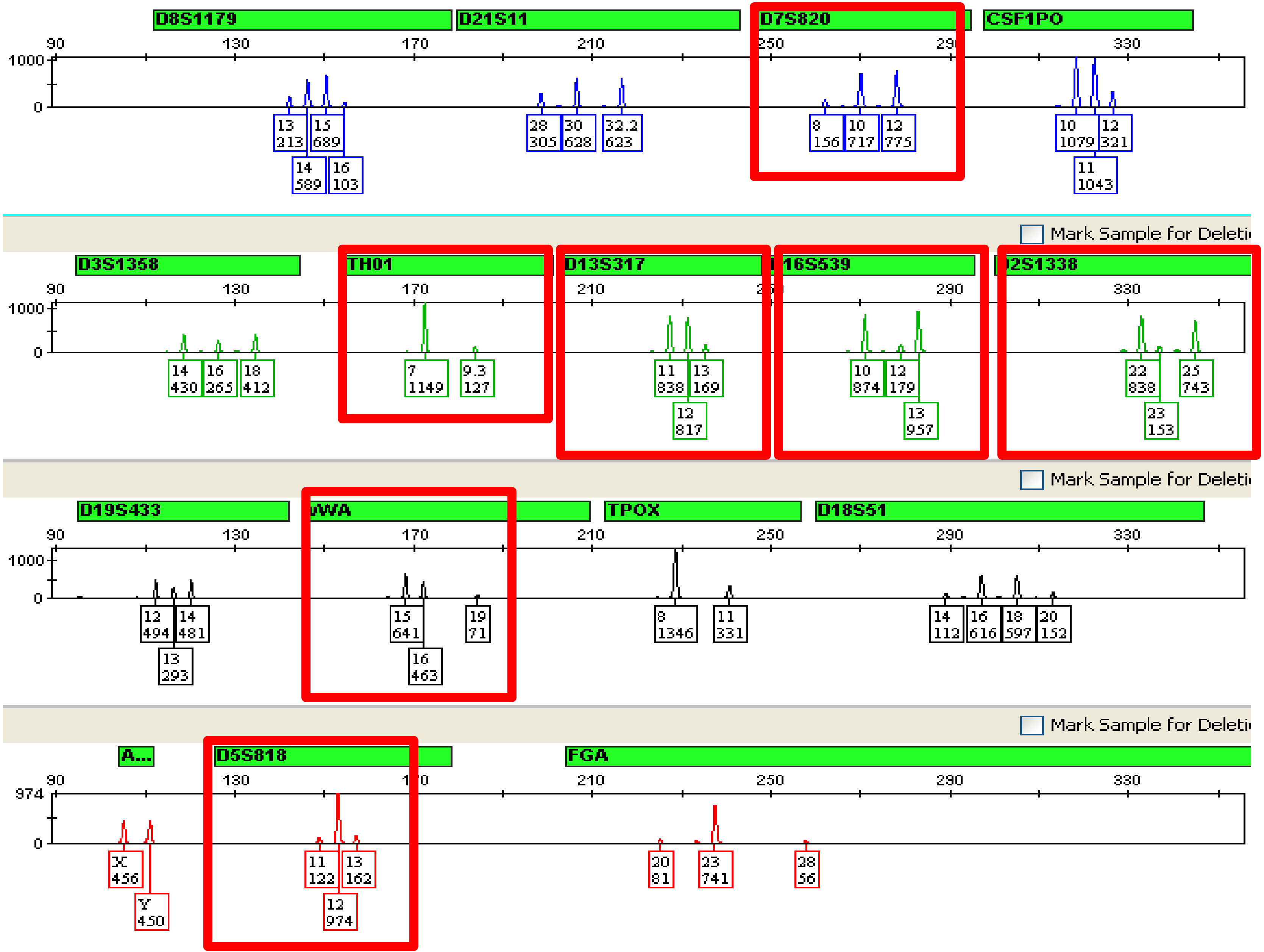
RMP = 2pq + $(q^2 + q(1-q)\theta)$

RMP = 2(0.5443)(0.2537) + $(0.2537)^2$ + (0.2537)(0.7463)(0.01)

= 0.3424 or 1 in 2.9

RMP/LR

Profile 1: ID_2_SCD_NG0.5_R4,1_A1_V1.2



If RMP/LR Stats are Used

Can use

D8

D21

D18

D3

D19

TPOX

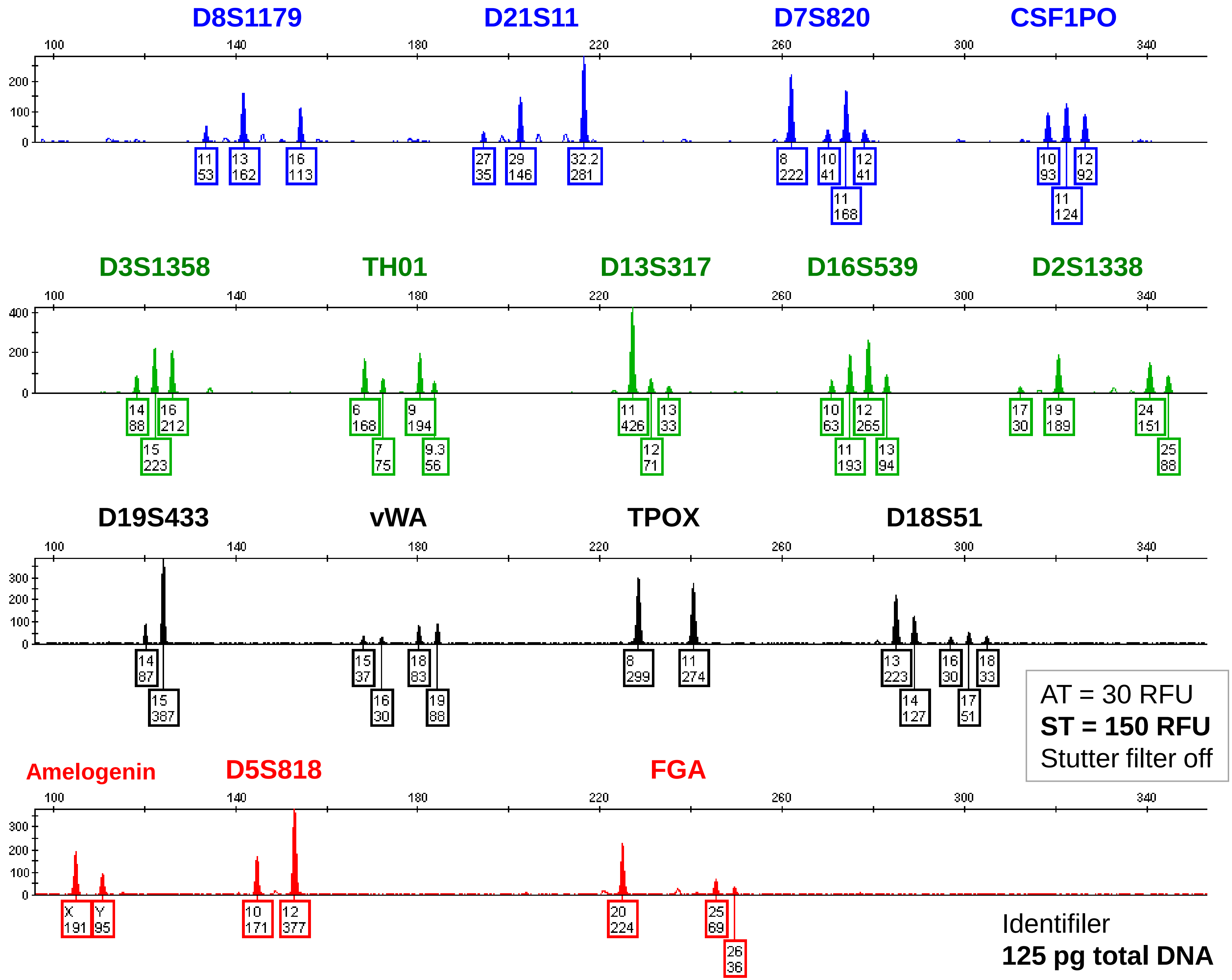
FGA

CSF

Loci with potential D-out

D7	D2
TH01	vWA
D13	D5
D16	

Challenges with low level, complex mixtures



D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338
D19S433
vWA
TPOX
D18S51
Amelogenin
D5S818
FGA
AT = 30 RFU
ST = 150 RFU
Stutter filter off
Identifiler
125 pg total DNA

Impact of Results with Low Level DNA

Clayton et al. (1998)
ISFG (2006) Rec. #4

Step #1
Identify the Presence of a Mixture

Step #2
Designate Allele Peaks

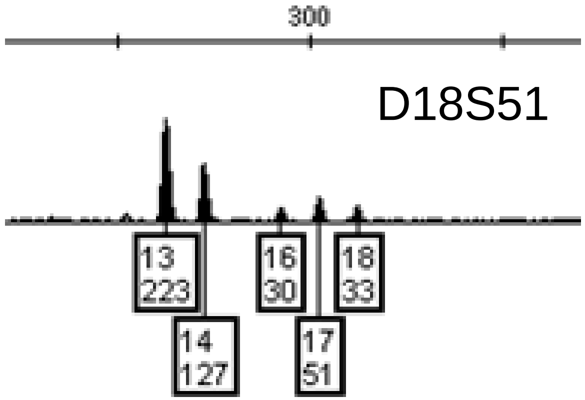
Step #3
Identify the Number of Potential Contributors

Step #4
Estimate the Relative Ratio of Contributors

Step #5
Consider All Possible Genotype Combinations

Step #6
Compare Reference Samples

When amplifying low amounts of DNA (e.g., 125 pg), allele dropout is a likely possibility leading to **higher uncertainty** in the potential number of contributors and in the possible genotype combinations



Identifiler

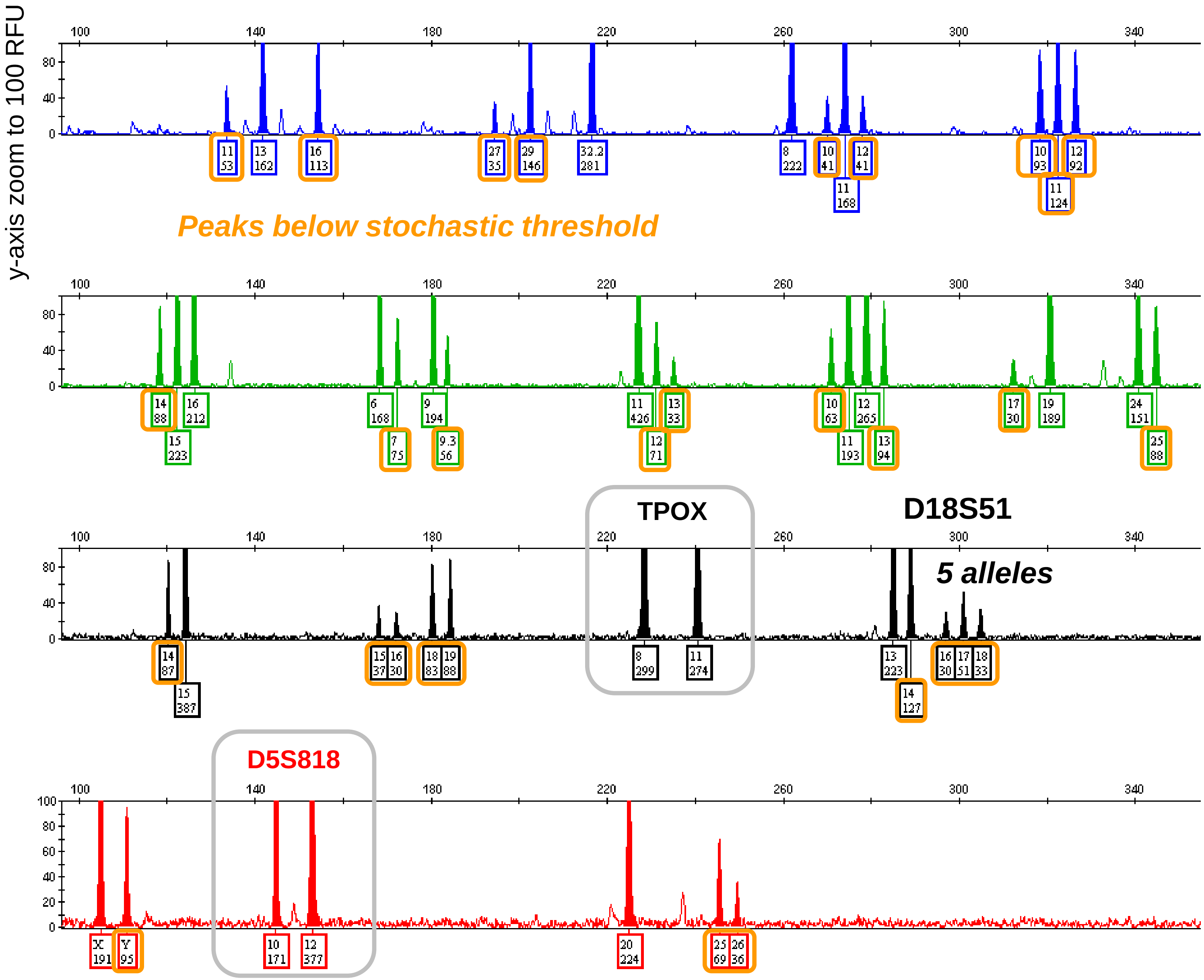
125 pg total DNA

Complex Mixture

AT = 30 RFU

ST = 150 RFU

Stutter filter off

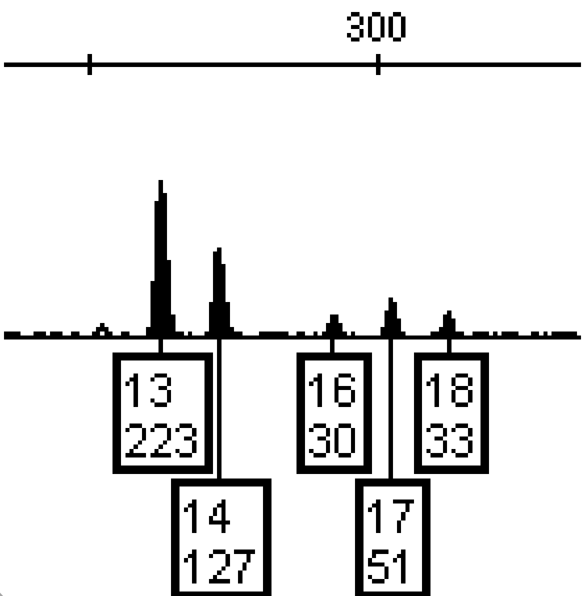


What Can We Say about this Result?

- Low level DNA (only amplified 125 pg total DNA)
  - likely to exhibit stochastic effects and have allele dropout
- Mixture of at least 3 contributors
  - Based on detection of 5 alleles at D18S51
  - If at equal amounts, ~40 pg of each contributor (if not equal, then less for the minor contributors); **we expect allele dropout**
- At least one of the contributors is male
  - Based on presence of Y allele at amelogenin
- Statistics if using CPI/CPE
  - Would appear that we can only use TPOX and D5S818 results with a stochastic threshold of 150 RFU *(will explore this further)*
- **Due to potential of excessive allele dropout, we are unable to perform any meaningful Q-K comparisons**

Uncertainty in the Potential Number of Contributors with this Result

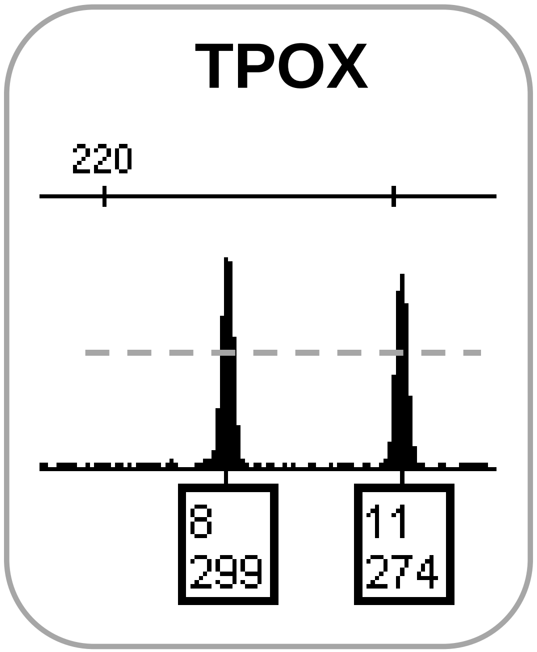
D18S51



5 alleles observed

- Several of the peaks are barely above the analytical threshold of 30 RFU
  - In fact, with an analytical threshold of 50 RFU or even 35 RFU, there would only be three detected alleles at D18S51
- Stochastic effects could result in a high degree of stutter off of the 17 allele making alleles 16 and 18 potential stutter products
- No other loci have >4 alleles detected

All Detected Alleles Are Above the Stochastic Threshold – Or Are They?



Stochastic threshold = 150 RFU

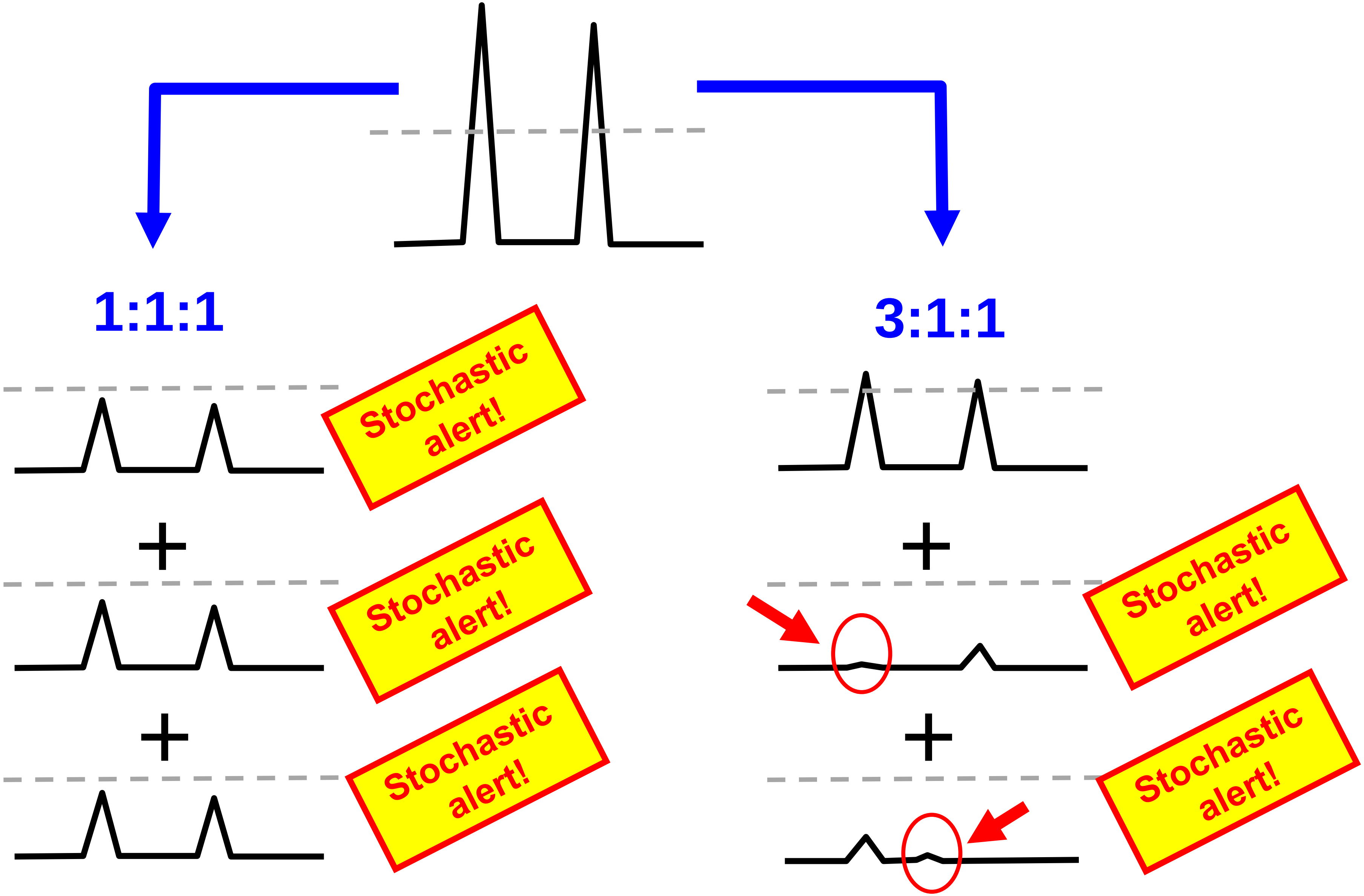
Does this result guarantee no allele drop-out?

We have assumed three contributors. If result is from an equal contribution of 3 individuals…

Then some alleles from individual contributors would be below the stochastic threshold and we could not assume that all alleles are being observed!

Assuming Three Contributors…

Some Possible Contributions to This Result

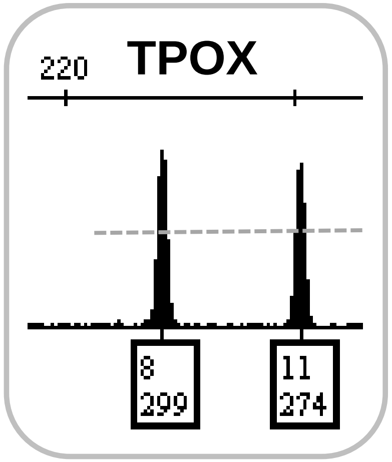


All Loci Are Not Created Equal
when it comes to mixture interpretation

- In the case of less polymorphic loci, such as TPOX, there are fewer alleles and these occur at higher frequency. Thus, there is a greater chance of allele sharing (peak height stacking) in mixtures.

- **Higher locus heterozygosity is advantageous for mixture interpretation** – we would expect to see more alleles (within and between contributors) and thus have a better chance of estimating the true number of contributors to the mixture

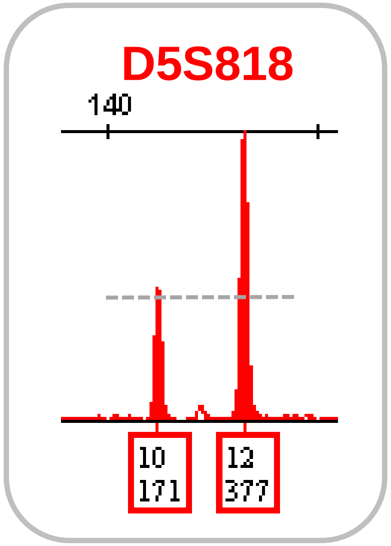
Even if you did attempt to calculate a CPI/CPE statistic using loci with all observed alleles above the stochastic threshold on this result…



<u>TPOX Allele Frequencies</u> (NIST Caucasian, Butler et al. 2003)
8 = 0.53
11 = 0.24
CPI = (0.53 + 0.24)^2 = 0.59 or **59%**

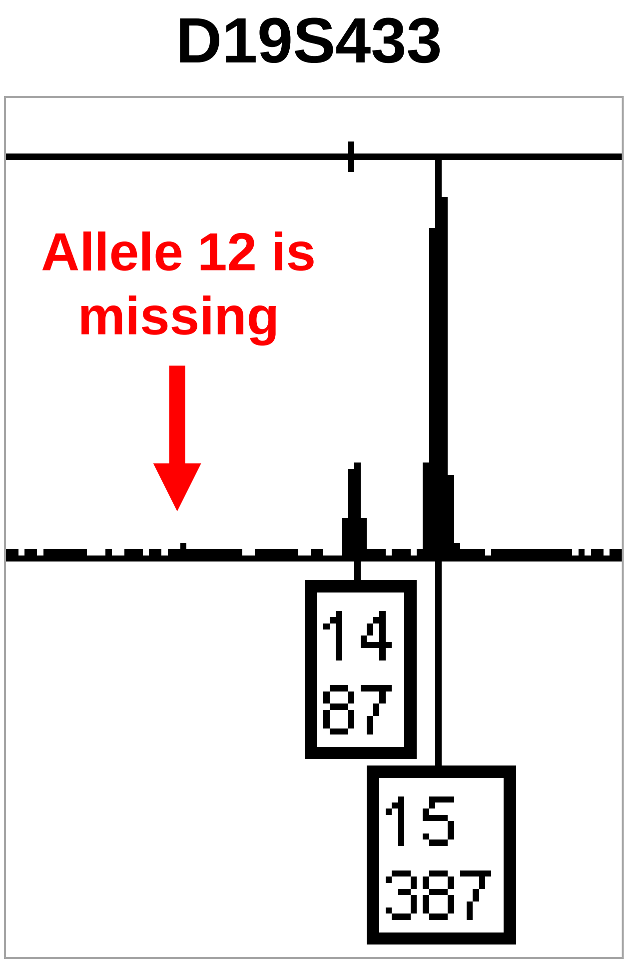
Combine loci = 0.59 x 0.18 = 0.11 or **11%**

Approximately 1 in every 9 Caucasians could be included in this mixture

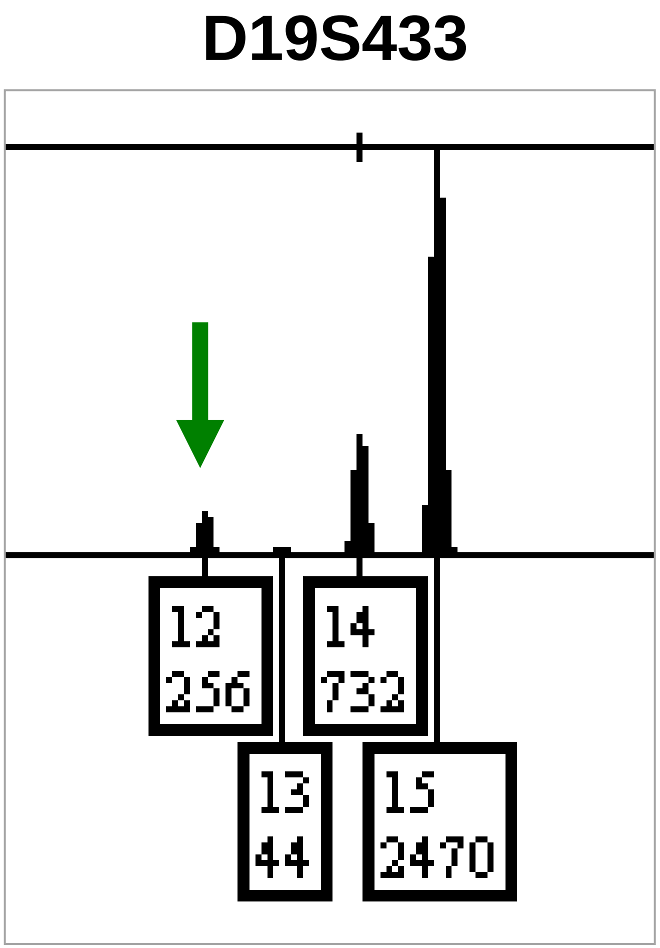


<u>D5S818 Allele Frequencies</u> (NIST Caucasian, Butler et al. 2003)
10 = 0.05
12 = 0.38
CPI = (0.05 + 0.38)^2 = 0.18 or **18%**

Impact of Amplifying More DNA



125 pg total DNA amplified



500 pg total DNA amplified

True Contributors

3 contributors with a 2:1:1 mixture

15,15 (2x)

14,15 (1x)

12,14 (1x)

How should you handle the suspect comparison(s) with this case result?

- **No suspect comparisons should be made** as **the mixture result has too much uncertainty** with stochastic effects that may not account for all alleles being detected

- **Declare the result "inconclusive"**

How not to handle this result

- “To heck with the analytical and stochastic thresholds”, **I am just going to see if the suspect profile(s) can fit into the mixture allele pattern observed** – and then if an allele is not present in the evidentiary sample try to explain it with possible allele dropout due to stochastic effects

- This is what Bill Thompson calls “painting the target around the arrow (matching profile)…”

Thompson, W.C. (2009) Painting the target around the matching profile: the Texas sharpshooter fallacy in forensic DNA interpretation. *Law, Probability and Risk* 8: 257-276

What to do with low level DNA mixtures?

- **German Stain Commission "Category C"** (Schneider et al. 2006, 2009)
  - Cannot perform stats because stochastic effects make it uncertain that all alleles are accounted for

- **ISFG Recommendations #8 & #9** (Gill et al. 2006)
  - Stochastic effects limit usefulness

- *Fundamentals of Forensic DNA Typing* (2010) Butler 3rd edition (volume 1), chapter 18
  - Don't go "outside the box" without supporting validation

ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
2. Scientists should be trained in and use LRs
3. Methods to calculate LRs of mixtures are cited
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
7. Allele dropout to explain evidence can only be used with low signal data
8. No statistical interpretation should be performed on alleles below threshold
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

A Complexity/Uncertainty Threshold

New Scientist article (August 2010)

- **How DNA evidence creates victims of chance**
  - 18 August 2010 by Linda Geddes
- From the last paragraph:
  - **In really complex cases, analysts need to be able to draw a line** and say "This is just too complex, I can't make the call on it," says Butler. "Part of the challenge now, is that every lab has that line set at a different place. But the honest thing to do as a scientist is to say: **I'm not going to try to get something that won't be reliable**."

http://www.newscientist.com/article/mg20727743.300-how-dna-evidence-creates-victims-of-chance.html

Is there a way forward?

“On the Threshold of a Dilemma”

- Gill and Buckleton (2010)
- Although most labs use thresholds of some description, this philosophy has always been problematic because there is an inherent illogicality which we call the falling off the cliff effect.

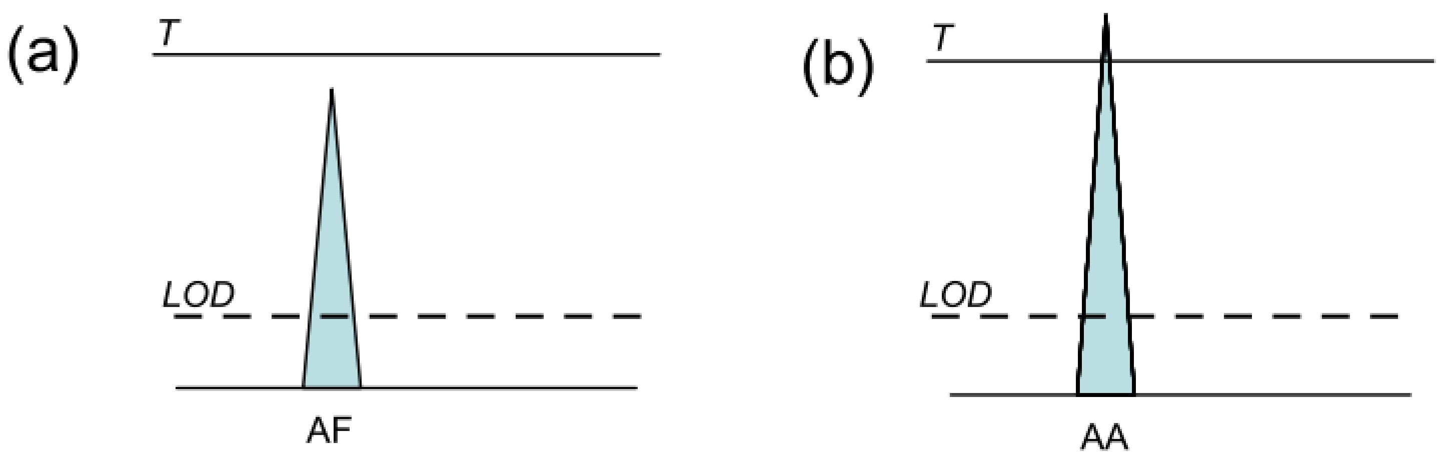
JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, January 2010, Vol. 55, No. 1
doi: 10.1111/j.1556-4029.2009.01257.x
Available online at: interscience.wiley.com

Commentary on: Budowle B, Onorato AJ, Callaghan TF, Della Manna A, Gross AM, Guerrieri RA, Luttman JC, McClure DL. Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. J Forensic Sci 2009;54(4):810–21.

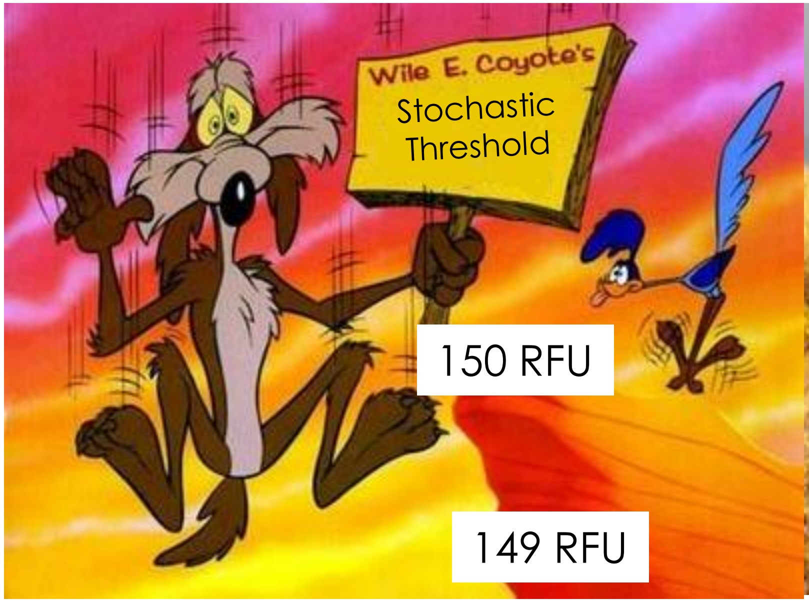
"Falling off the Cliff Effect"

- If T = an arbitrary level (e.g., 150 rfu), an allele of 149 rfu is subject to a different set of guidelines compared with one that is 150 rfu even though they differ by just 1 rfu (Fig. 1).



Gill and Buckleton *JFS* 55: 265-268 (2010)

Falling off the Cliff vs. Gradual Decline



http://blog.sironaconsulting.com/.a/6a00d8341c761a53ef011168cc5ff3970c-pi

http://ultimateescapesdc.files.wordpress.com/2010/08/mountainbiking2.jpg

Gill and Buckleton *JFS* **55:** 265-268 (2010)

- “The purpose of the ISFG DNA commission document was to provide a way forward to demonstrate the use of ***probabilistic models to circumvent the requirement for a threshold*** and to safeguard the legitimate interests of defendants.”

JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, 2011
doi: 10.1111/j.1556-4029.2011.01859.x
Available online at: onlinelibrary.wiley.com

PAPER

CRIMINALISTICS

Mark W. Perlin,[1] M.D., Ph.D.; Matthew M. Legler,[1] B.S.; Cara E. Spencer,[1] M.S.; Jessica L. Smith,[1] M.S.; William P. Allan,[1] M.S.; Jamie L. Belrose,[2] M.S.; and Barry W. Duceman,[3] Ph.D.

Validating TrueAllele® DNA Mixture Interpretation*,†

- Quantitative computer interpretation using
  Markov Chain Monte Carlo testing
- Models peak uncertainty and infers possible genotypes
- Results are presented as the Combined LR

True Allele Software (Cybergenetics)

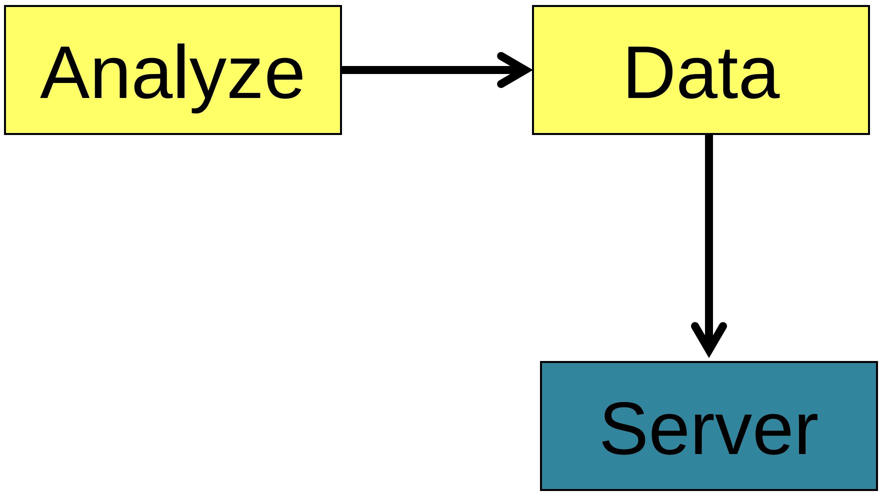
- We purchased the software in September 2010.
- Three day training at Cybergenetics (Pittsburgh, PA) in October.
- Software runs on a Linux Server with a Mac interface.

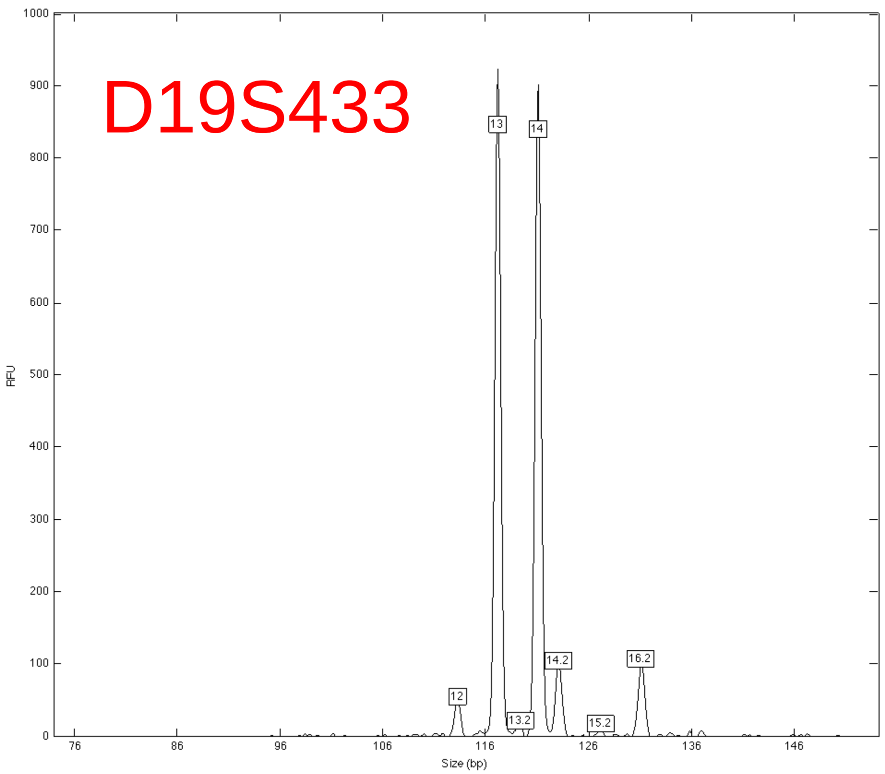
True Allele Casework Workflow
5 Modules

Analyze

.fsa files imported
Size Standard check
Allelic Ladder check
Alleles are called

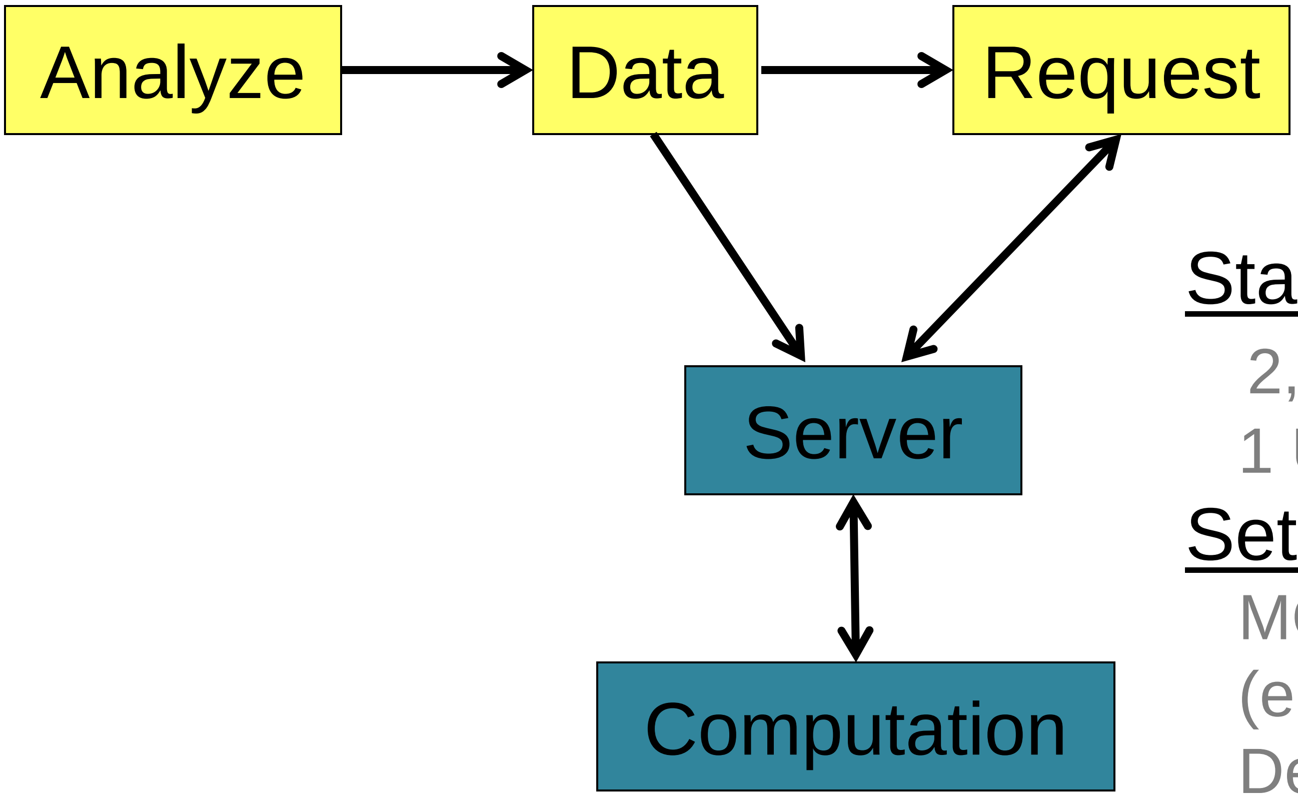
True Allele Casework Workflow
5 Modules





All Peaks above 10 RFU are considered

True Allele Casework Workflow
5 Modules



<u>State Assumptions</u>

2, 3, 4 unknowns

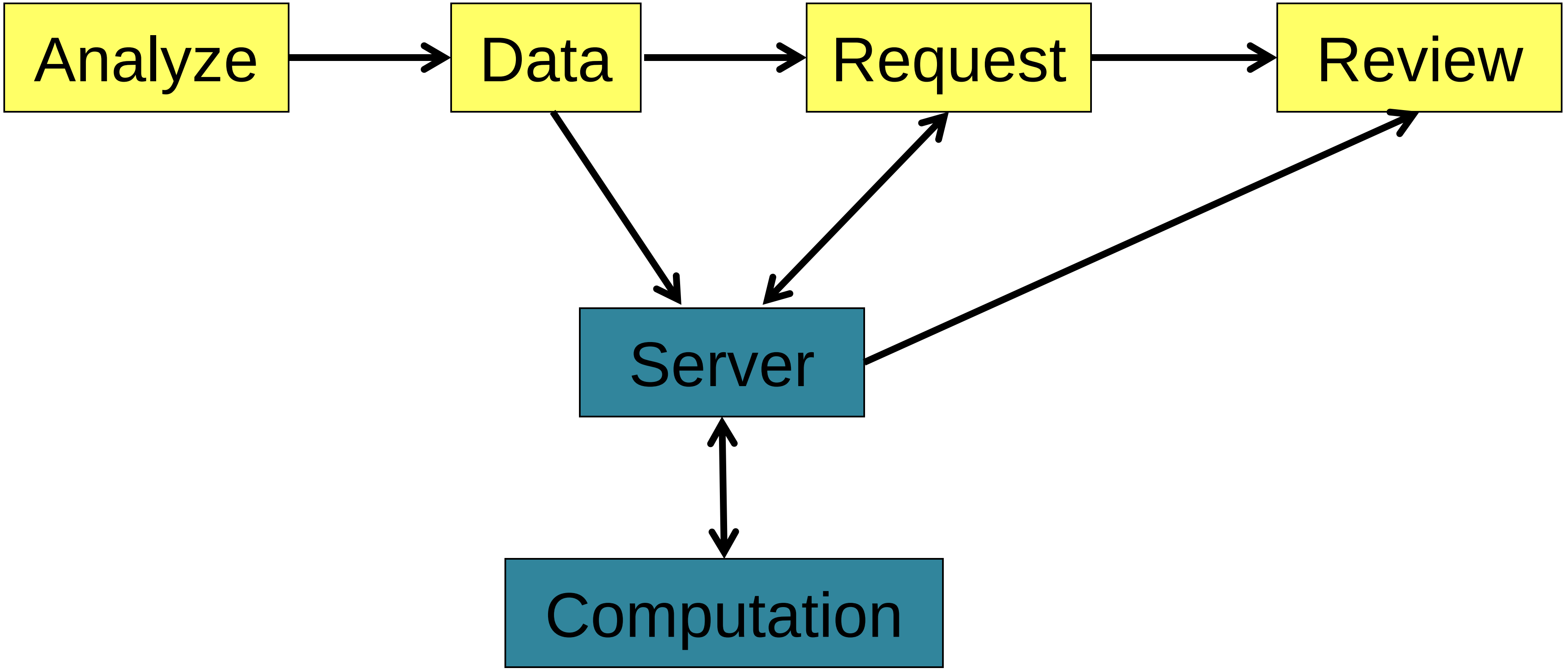
1 Unk with Victim?

<u>Set Parameters</u>

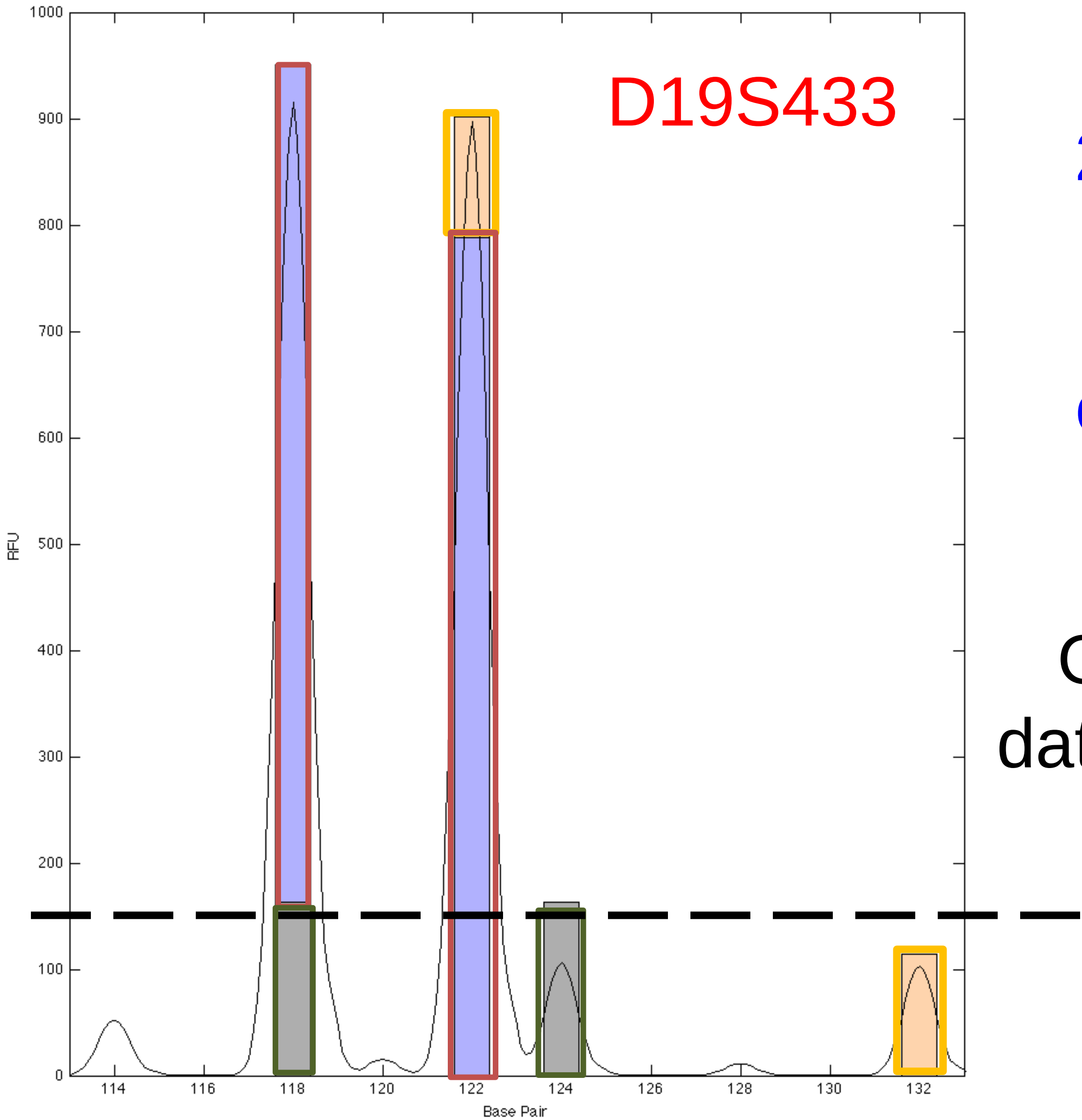
MCMC modeling (e.g.50K)

Degradation?

True Allele Casework Workflow
5 Modules



Review of One Replicate (of 50K)

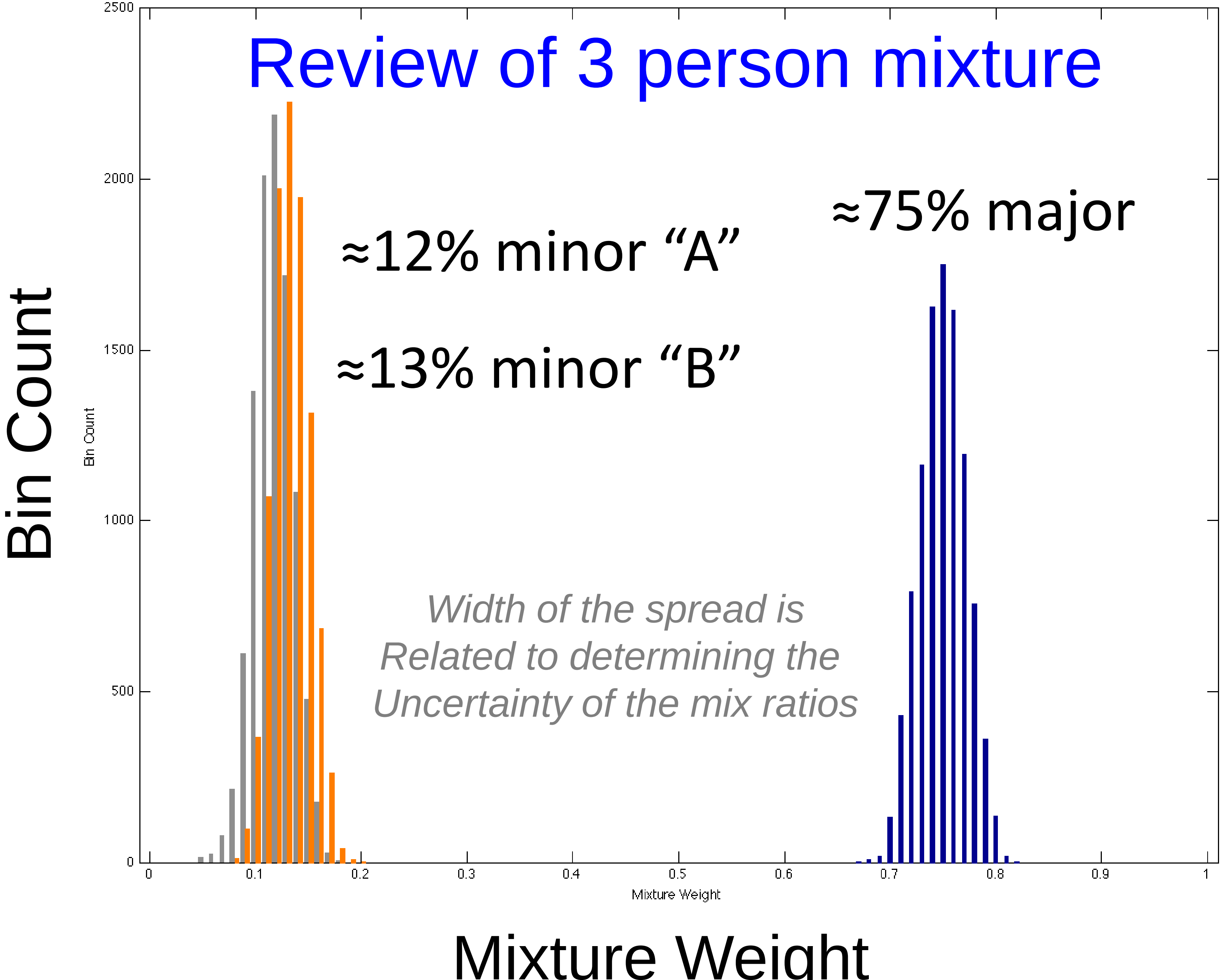


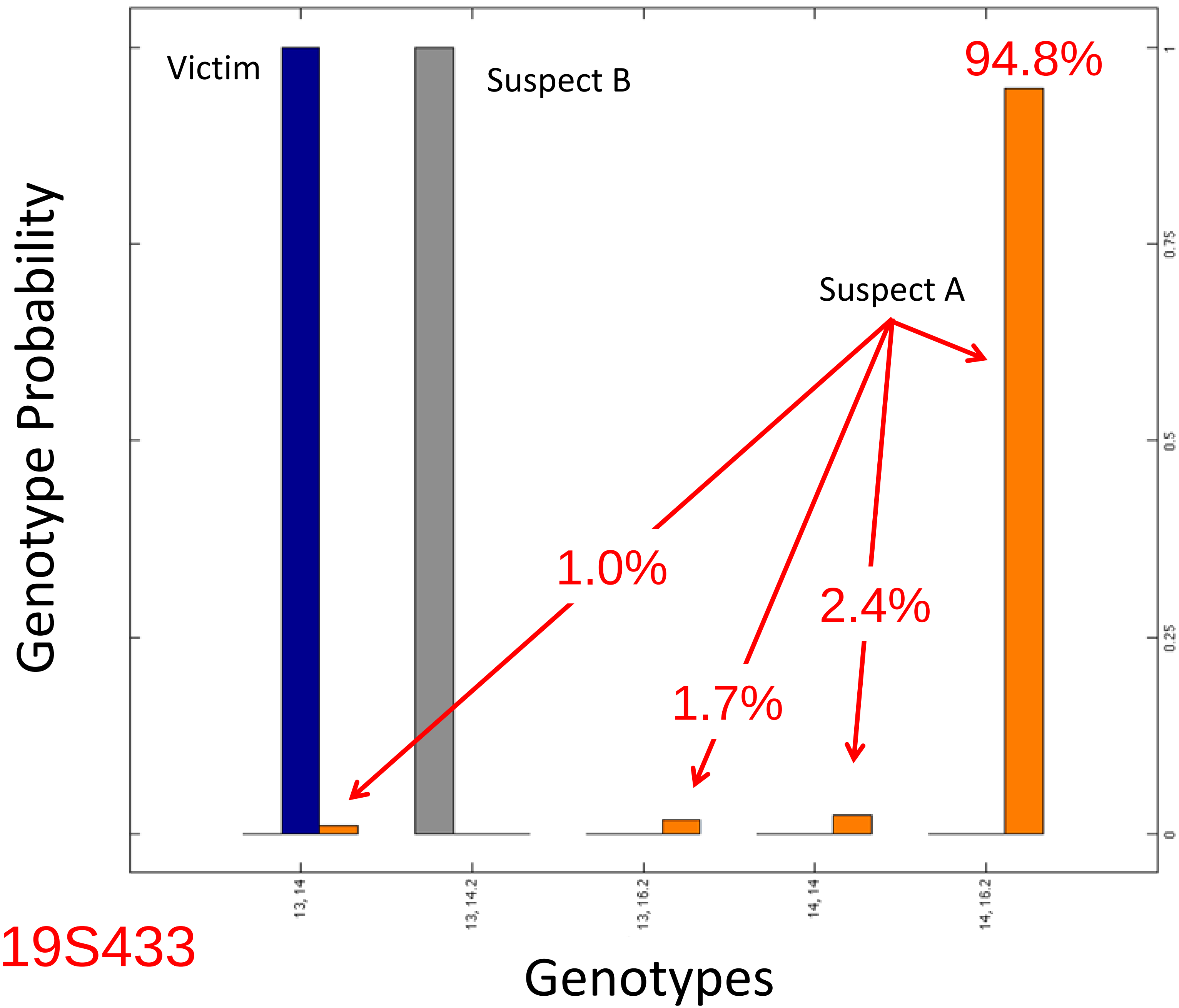
3P mixture,
2 Unknowns,

Conditioned on the Victim (major)

Good fit of the data to the model

Review of 3 person mixture

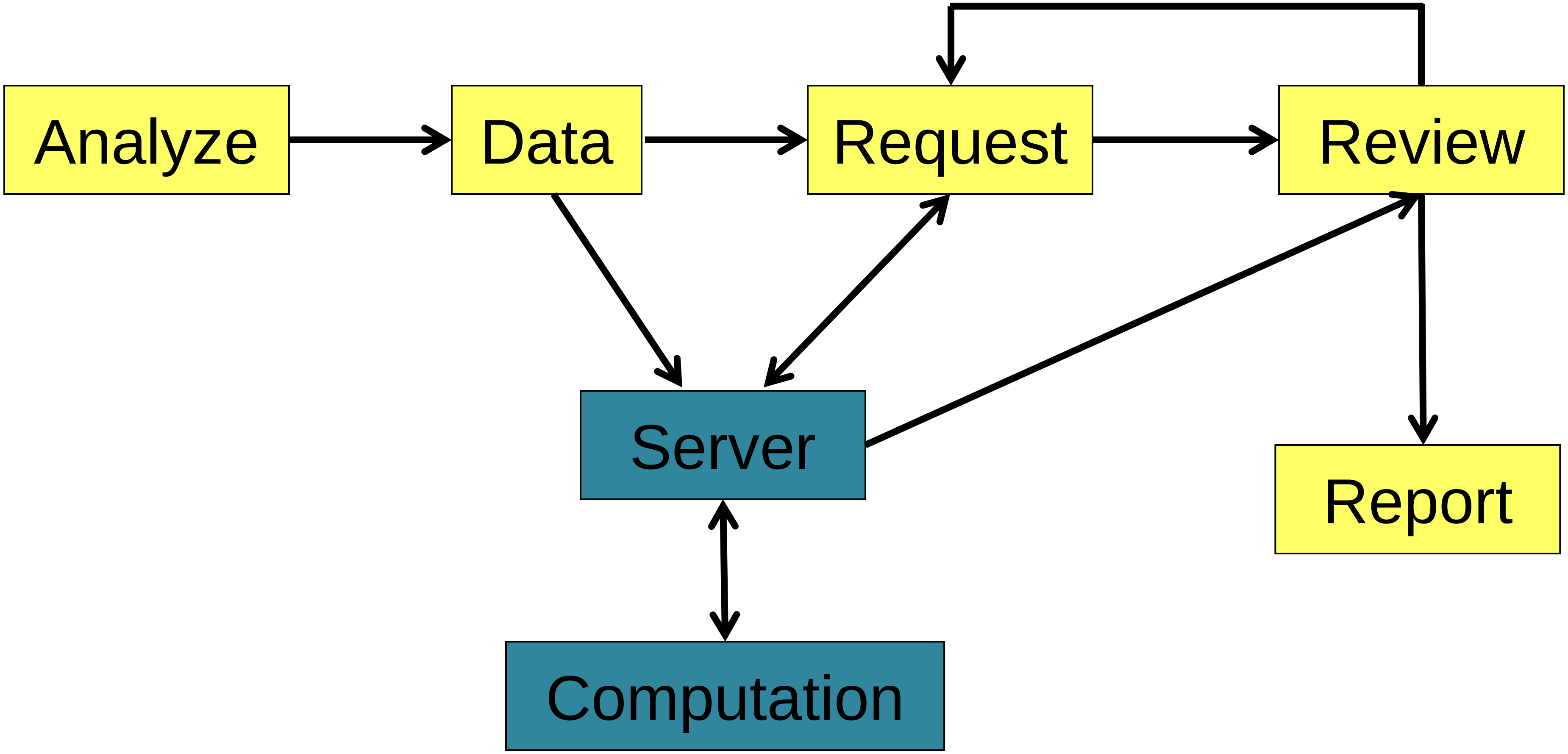




Genotype Probability
Victim
Suspect B
Suspect A
94.8%
1.0%
1.7%
2.4%
0
0.25
0.5
0.75
1
13, 14
13, 14.2
13, 16.2
14, 14
14, 16.2
Genotypes

D19S433

True Allele Casework Workflow
5 Modules



Determining the LR for D19S433

Suspect A = 14, 16.2

$H_P = 0.967$

Allele Pair	Probability Before Conditioning
14, 16.2	0.967
14, 14	0.003
13, 16.2	0.026
13, 14	0.001

$$LR = \frac{0.967}{}$$

Determining the LR for D19S433

Suspect A = 14, 16.2

$H_P = 0.967$

Allele Pair	Probability Before Conditioning	Genotype Frequency	Probability * Genotype Freq
14, 16.2	0.967	0.0120	0.01164
14, 14	0.003	0.0498	0.00013
13, 16.2	0.026	0.0131	0.00034
13, 14	0.001	0.1082	0.00009
		sum	**0.0122**

$$LR = \frac{0.967}{0.0122} = 79.26$$

H_D

Combined LR = 5.6 Quintillion

			Genotype Probability Distribution			Weighted Likelihood		Likelihood Ratio	
	allele pair	Likelihood	Questioned	Reference	Suspect	Numerator	Denominator	LR	log(LR)
locus	x	l(x)	q(x)	r(x)	s(x)	l(x)*s(x)	l(x)*r(x)		
CSF1PO	11, 12	0.686	0.778	0.1448	1	0.68615	0.1292	5.31	0.725
D13S317	9, 12	1	1	0.0291	1	0.99952	0.02913	34.301	1.535
D16S539	9, 11	0.985	0.995	0.1238	1	0.98451	0.12188	8.036	0.905
D18S51	13, 17	0.999	1	0.0154	1	0.99915	0.01543	64.677	1.811
D19S433	14, 16.2	0.967	0.948	0.012	1	0.96715	0.01222	79.143	1.898
D21S11	28, 30	0.968	0.98	0.0872	1	0.96809	0.08648	11.194	1.049
D2S1338	23, 24	0.998	1	0.0179	1	0.99831	0.01787	55.866	1.747
D3S1358	15, 17	0.988	0.994	0.1224	1	0.98759	0.12084	8.14	0.911
D5S818	11, 11	0.451	0.394	0.0537	1	0.45103	0.07309	6.17	0.79
D7S820	11, 12	0.984	0.978	0.0356	1	0.98383	0.03617	27.198	1.435
D8S1179	13, 14	0.203	0.9	0.1293	1	0.20267	0.02993	6.771	0.831
FGA	21, 25	0.32	0.356	0.028	1	0.31986	0.01906	16.783	1.225
TH01	7, 7	0.887	0.985	0.1739	1	0.88661	0.15588	5.687	0.755
TPOX	8, 8	1	1	0.1375	1	1	0.13746	7.275	0.862
vWA	15, 20	0.998	0.996	0.0057	1	0.99808	0.00569	174.834	2.243

Results

- Results are expressed as logLR values

$$LR = 1{,}000{,}000 = 10^6$$

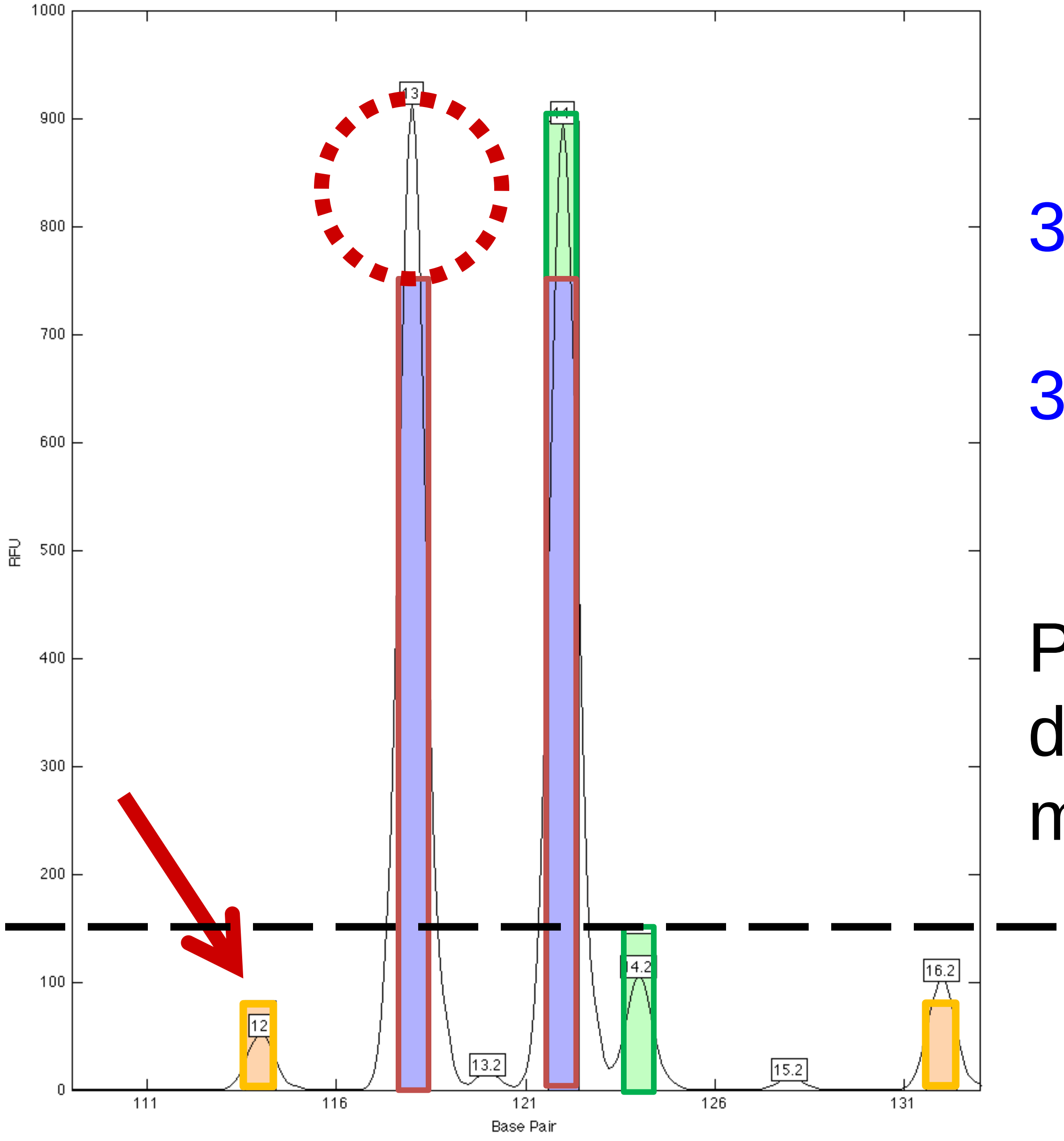
$$\log(LR) = \log 10^6$$

$$\log(LR) = 6 * \cancel{\log 10}\ (1)$$

$$\log(LR) = 6$$

Review of One Replicate (of 50K)

D19S433



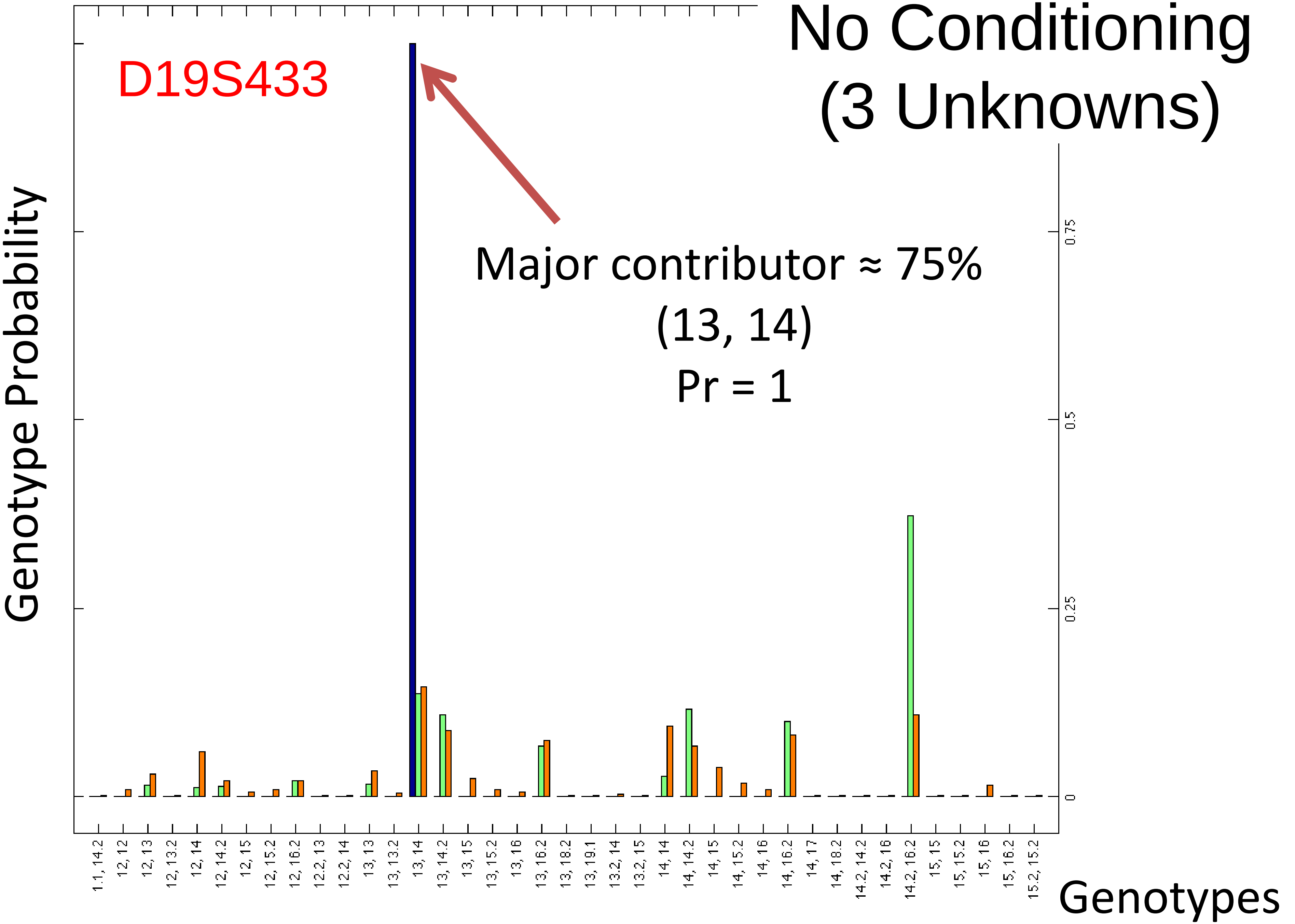
150 RFU

3P mixture,

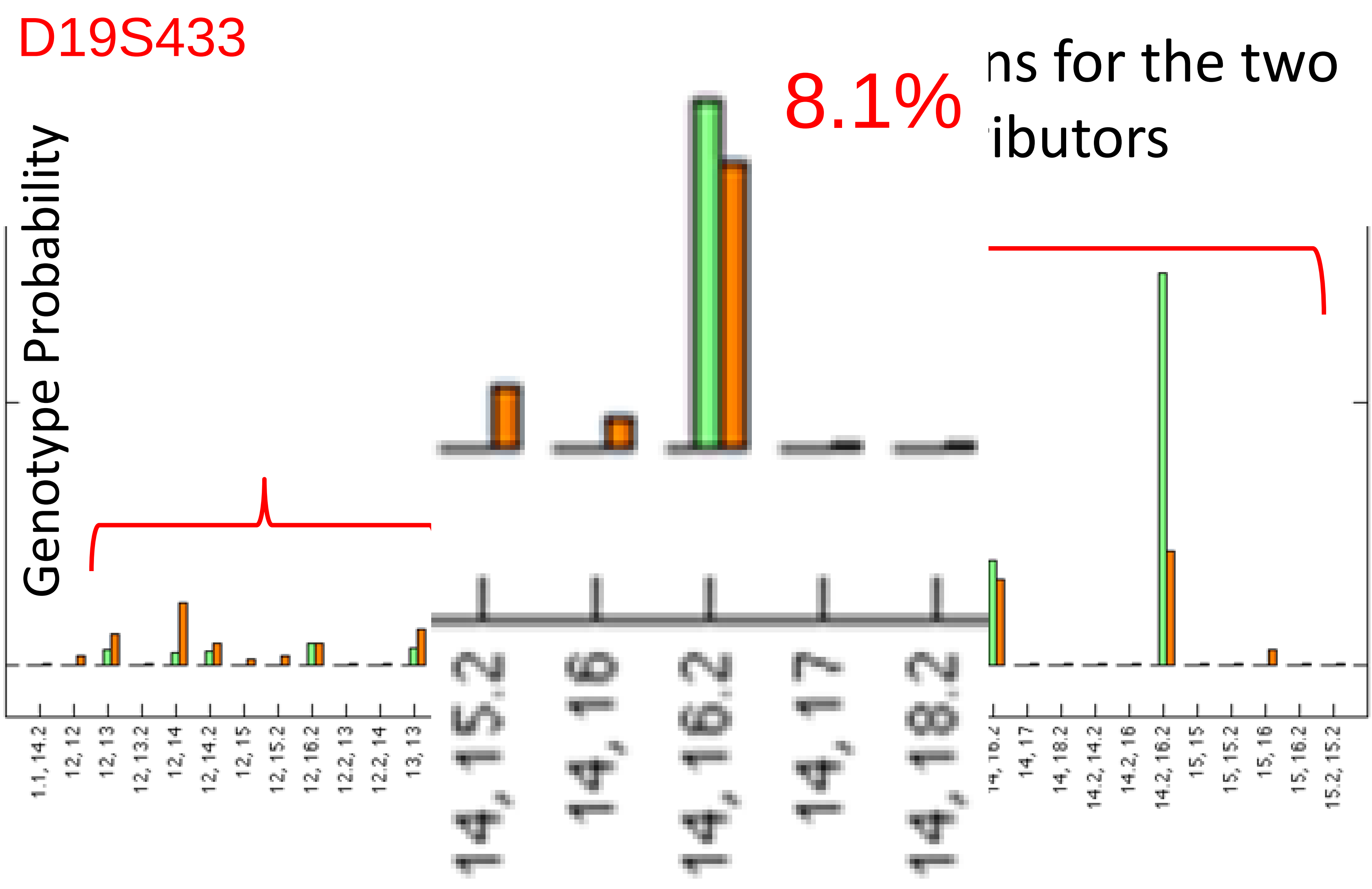
3 Unknowns

Poor fit of the data to the model

No Conditioning (3 Unknowns)



No Conditioning (3 Unknowns)



locus	allele pair	L	Q	R	S	L*S	L*R	LR	log(LR)
D19S433	13 , 14	0.002	0.146	0.1082			0.00020		
	14.2, 16.2	0.270	0.109	0.0044			0.00118		
	14 , 14	0.002	0.093	0.0498			0.00008		
	13 , 14.2	0.017	0.088	0.0392			0.00068		
	14 , 16.2	0.013	0.081	0.0120	1	0.01295	0.00016		
	13 , 16.2	0.018	0.074	0.0131			0.00023		
	14 , 14.2	0.009	0.067	0.0361			0.00031		
	12 , 14	0.002	0.059	0.0498			0.00012		
	14 , 15	0.001	0.038	0.0343			0.00002		
	13 , 13	0.001	0.034	0.0587			0.00007		
	12 , 13	0.002	0.029	0.0541			0.00010		
	13 , 15	0.001	0.024	0.0373			0.00002		
	12 , 16.2	0.017	0.021	0.0060			0.00010		
	12 , 14.2	0.013	0.020	0.0180			0.00023		
	14 , 15.2	0.001	0.018	0.0275			0.00003		
	15 , 16	0.002	0.015	0.0006			0.00000		
	13 , 15.2	0.001	0.009	0.0299			0.00003		
	12 , 15.2	0.003	0.009	0.0137			0.00004		
	14 , 16	0.000	0.009	0.0017			0.00000		
	12 , 12	0.004	0.009	0.0125			0.00004		
	12 , 15	0.001	0.006	0.0172			0.00001		
	13 , 16	0.000	0.006	0.0019			0.00000		
	13 , 13.2	0.001	0.004	0.0261			0.00003		
	13.2, 14	0.001	0.003	0.0240			0.00002		
	13.2, 15	0.001	0.002	0.0083			0.00001		
	14 , 18.2	0.002	0.002	0.0017			0.00000		
	13 , 19.1	0.019	0.002	0.0000			0.00000		
	12 , 13.2	0.002	0.002	0.0120			0.00003		
	14.2, 16	0.001	0.002	0.0006			0.00000		
	12.2, 13	0.001	0.002	0.0168			0.00002		
	13 , 18.2	0.002	0.001	0.0019			0.00000		
	12.2, 14	0.001	0.001	0.0155			0.00001		
	14.2, 14.2	0.004	0.001	0.0065			0.00003		
	15 , 15	0.000	0.001	0.0059			0.00000		
	15 , 15.2	0.000	0.001	0.0095			0.00000		
	14 , 17	0.001	0.001	0.0000			0.00000		
	15 , 16.2	0.000	0.001	0.0042			0.00000		
	15.2, 15.2	0.001	0.001	0.0038			0.00000		
	1.1, 14.2	0.072	0.001	0.0097			0.00069		
						0.01295	0.00385	3.367	0.527

Suspect “A” Genotype

39 probable genotypes

D19S433

Suspect A = 14, 16.2 $H_P = 0.013$

Allele Pair	Probability	Genotype Frequency		Prob * GenFreq
13,14	0.002	0.1082		0.00020
14.2, 16.2	0.270	0.0044		0.00118
14, 14	0.002	0.0498		0.00008
13, 14.2	0.017	0.0392		0.00068
14, 16.2	0.013	0.0120		0.00016
13, 16.2	0.018	0.0131		0.00023
etc…	etc…	etc…		etc…
			Sum	**0.00385**

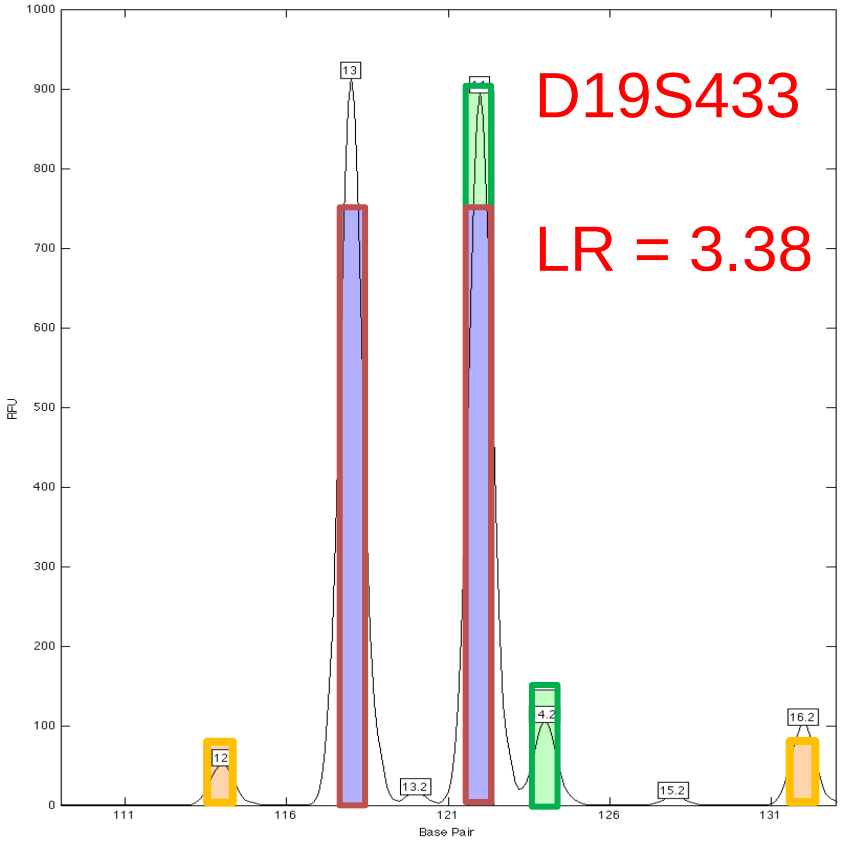
H_D

$$LR = \frac{0.013}{0.00385} = 3.38$$

D19S433

No Conditioning (3 Unknowns)

No Conditioning

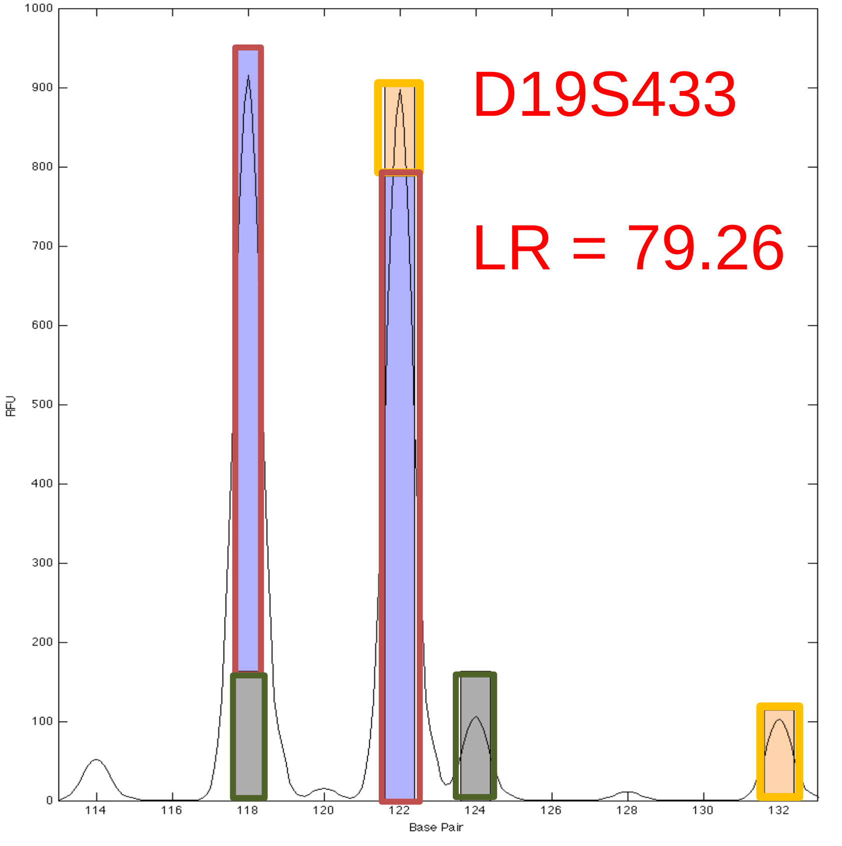


Profile - Combined log(LR)

Suspect A log(LR) = 8.03

Suspect B log(LR) = 7.84

Conditioned on Victim



Profile - Combined log(LR)

Suspect A log(LR) = 18.72

Suspect B log(LR) = 19.45

Exploring the Capabilities

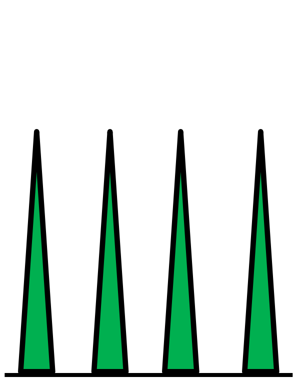
- **Degree of Allele Sharing**
- **Mixture Ratios**
- DNA Quantity

Mixture Data Set

- Mixtures of pristine male and female DNA amplified at a total concentration of 1.0 ng/μL using Identifiler (standard conditions).
- Mixture ratios ranged from 90:10, 80:20, 70:30 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90
- Each sample was amplified twice.

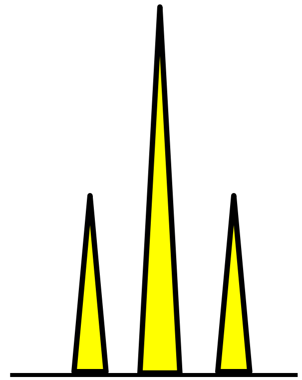
Mixture Data Set

- Three different combinations:



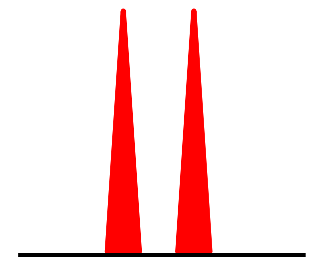
"Low" Sharing

4 alleles – 10 loci
3 alleles – 5 loci
2 alleles – 0 loci
1 allele – 0 loci



"Medium" Sharing

4 alleles – 3 loci
3 alleles – 8 loci
2 alleles – 4 loci
1 allele – 0 loci

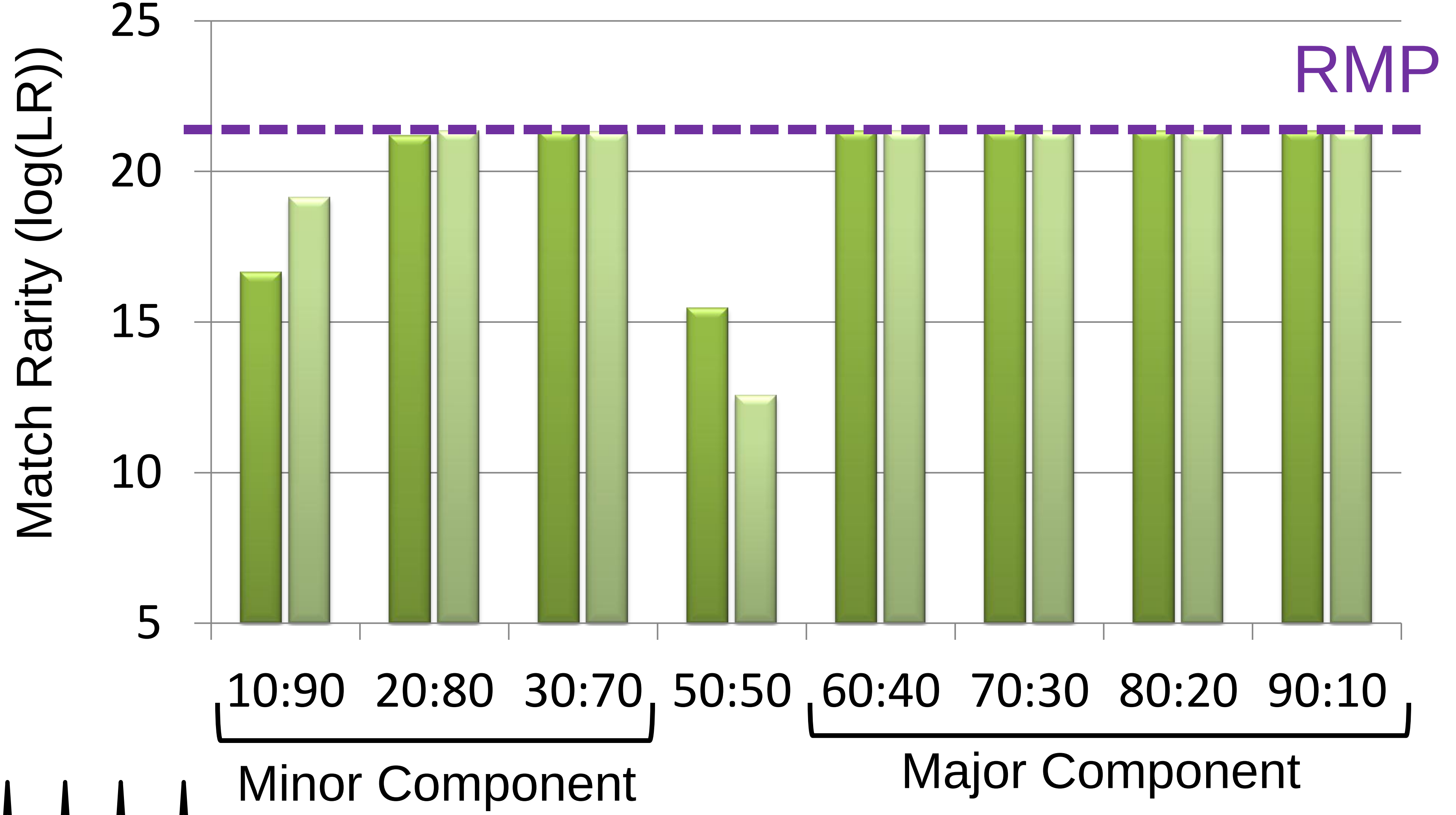


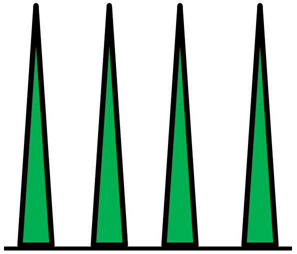
"High" Sharing

4 alleles – 0 loci
3 alleles – 6 loci
2 alleles – 8 loci
1 allele – 1 loci

Virtual MixtureMaker - http://www.cstl.nist.gov/strbase/software.htm

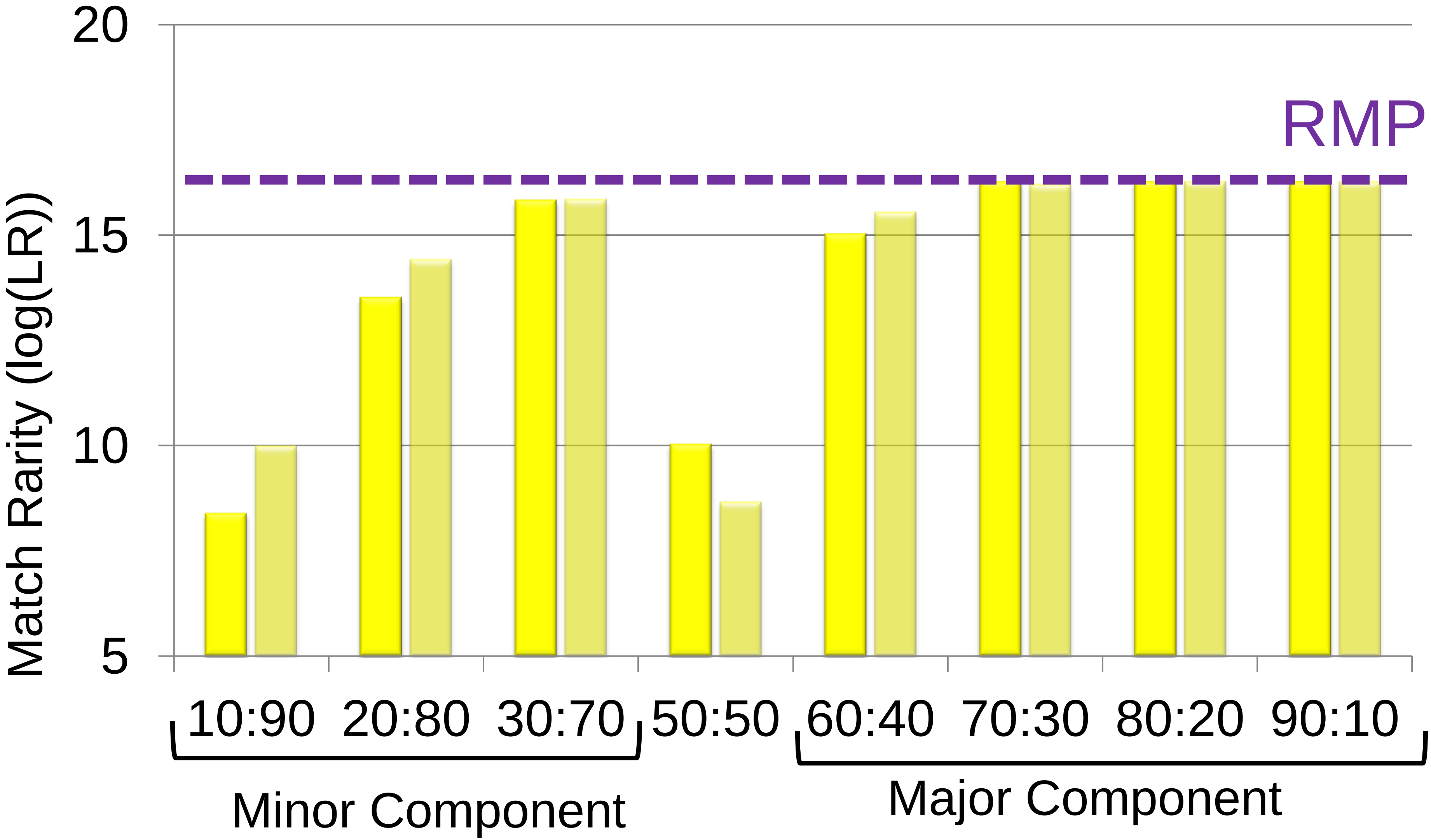
Match Score in Duplicate Runs

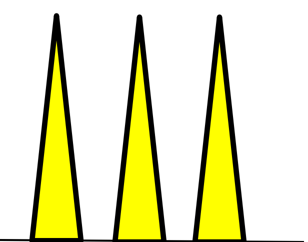




“Easy” for Deconvolution

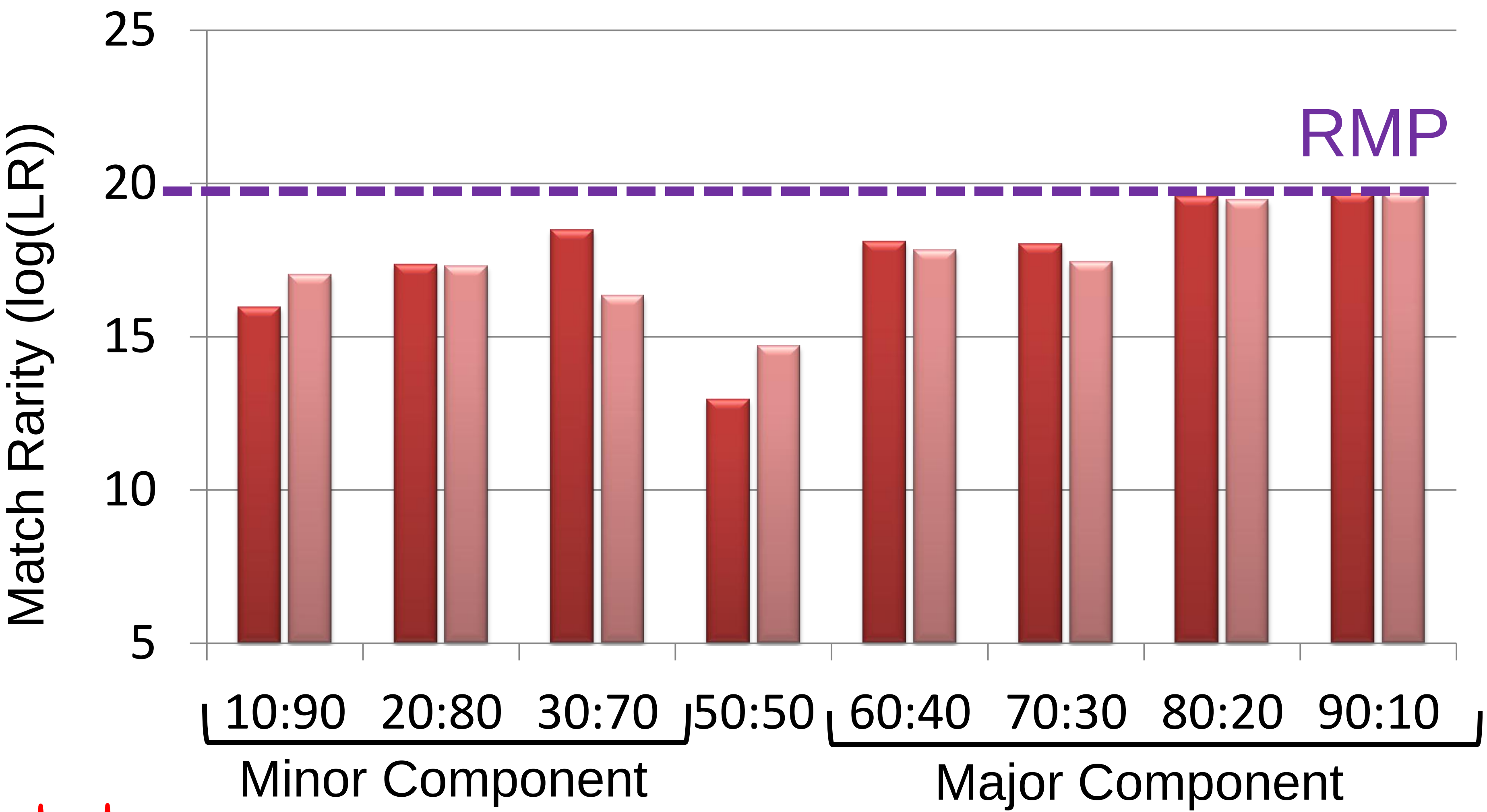
Match Score in Duplicate Runs

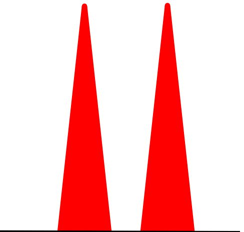




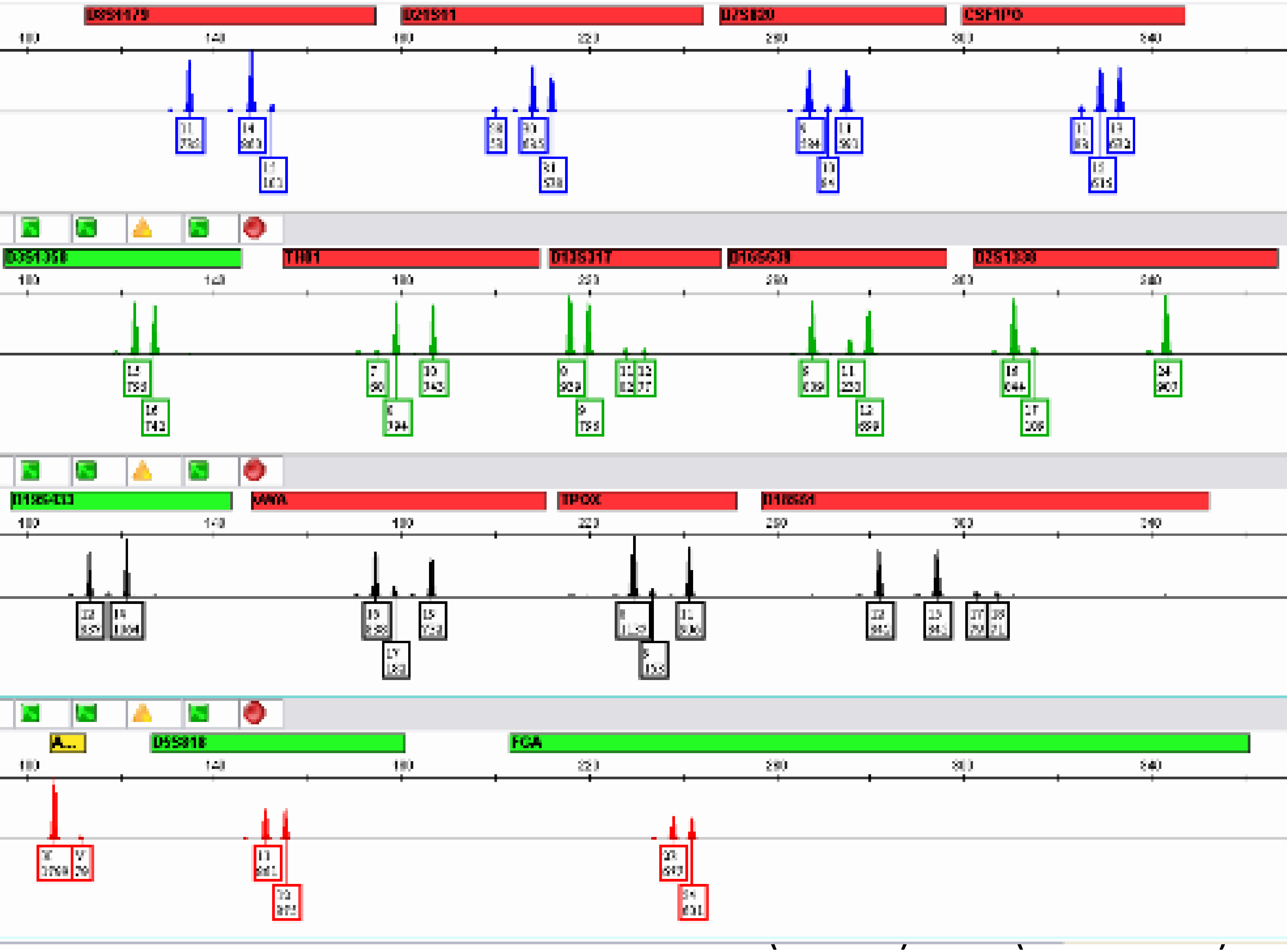
“Challenging” for Deconvolution

Match Score in Duplicate Runs

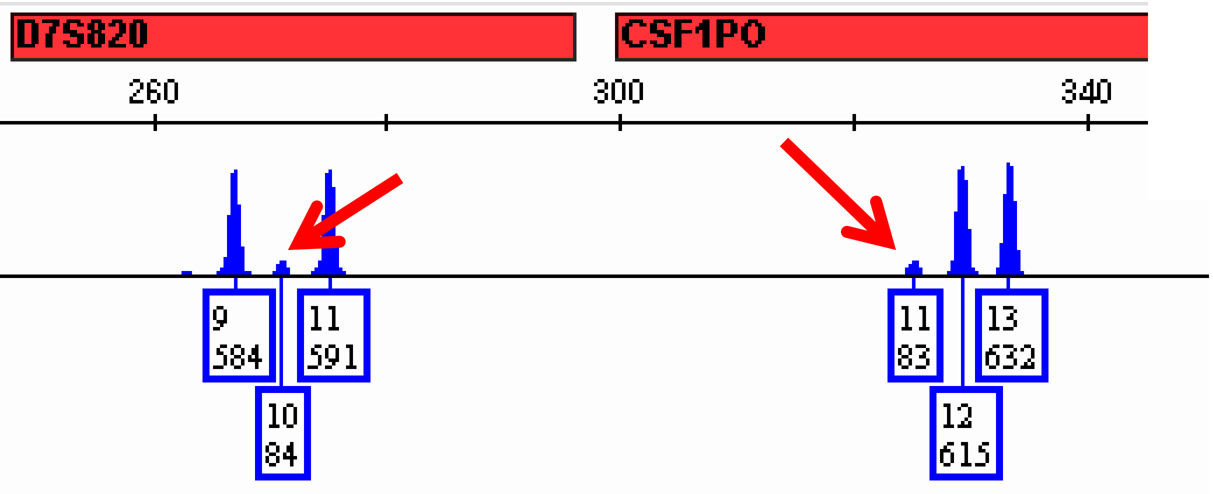




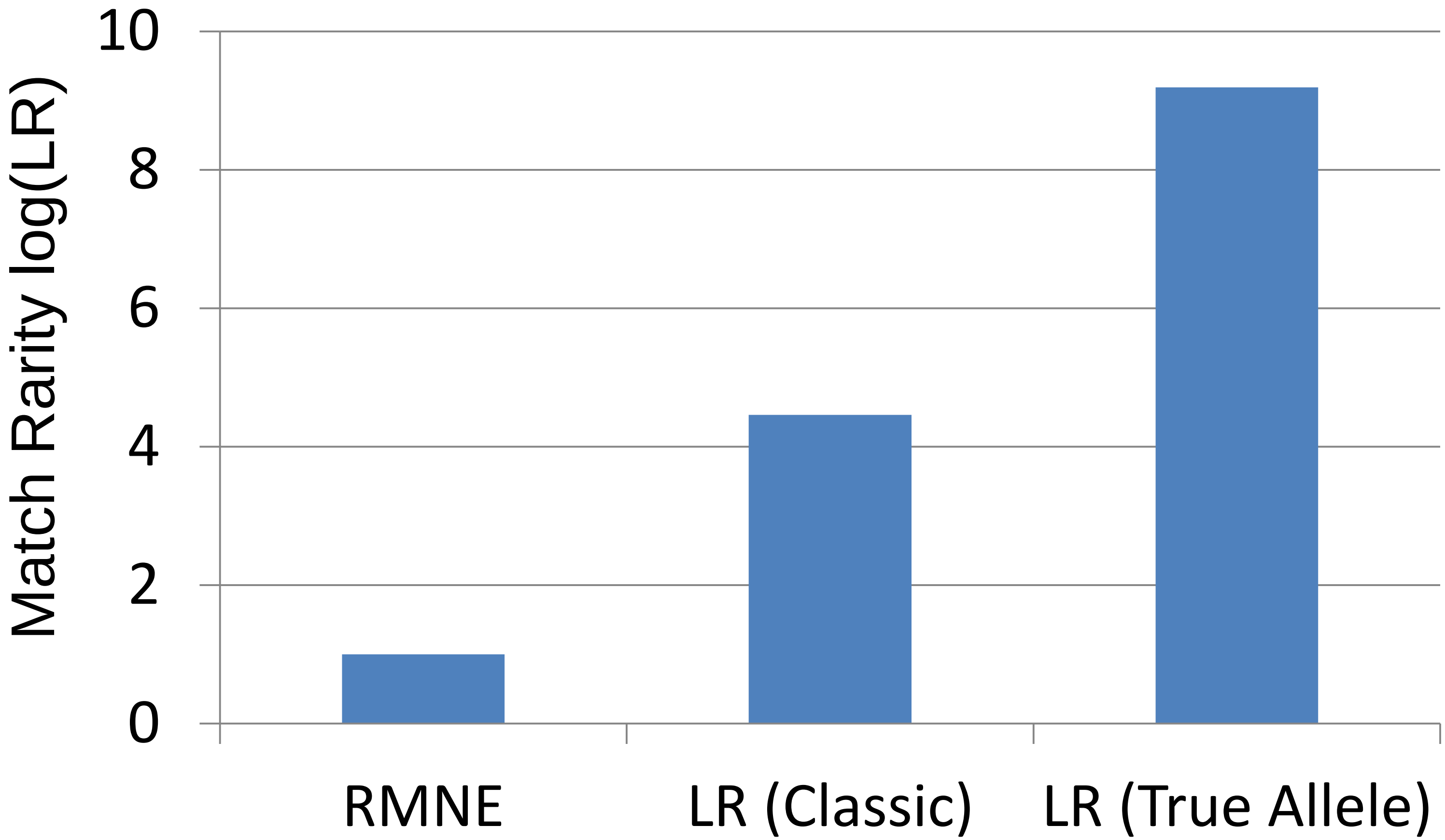
“Difficult” for Deconvolution



D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338
D19S433
vWA
TPOX
D18S51
A...
D5S818
FGA



10:90
minor contributor



Exploring the Capabilities

- Degree of Allele Sharing
- Mixture Ratios
- **DNA Quantity**

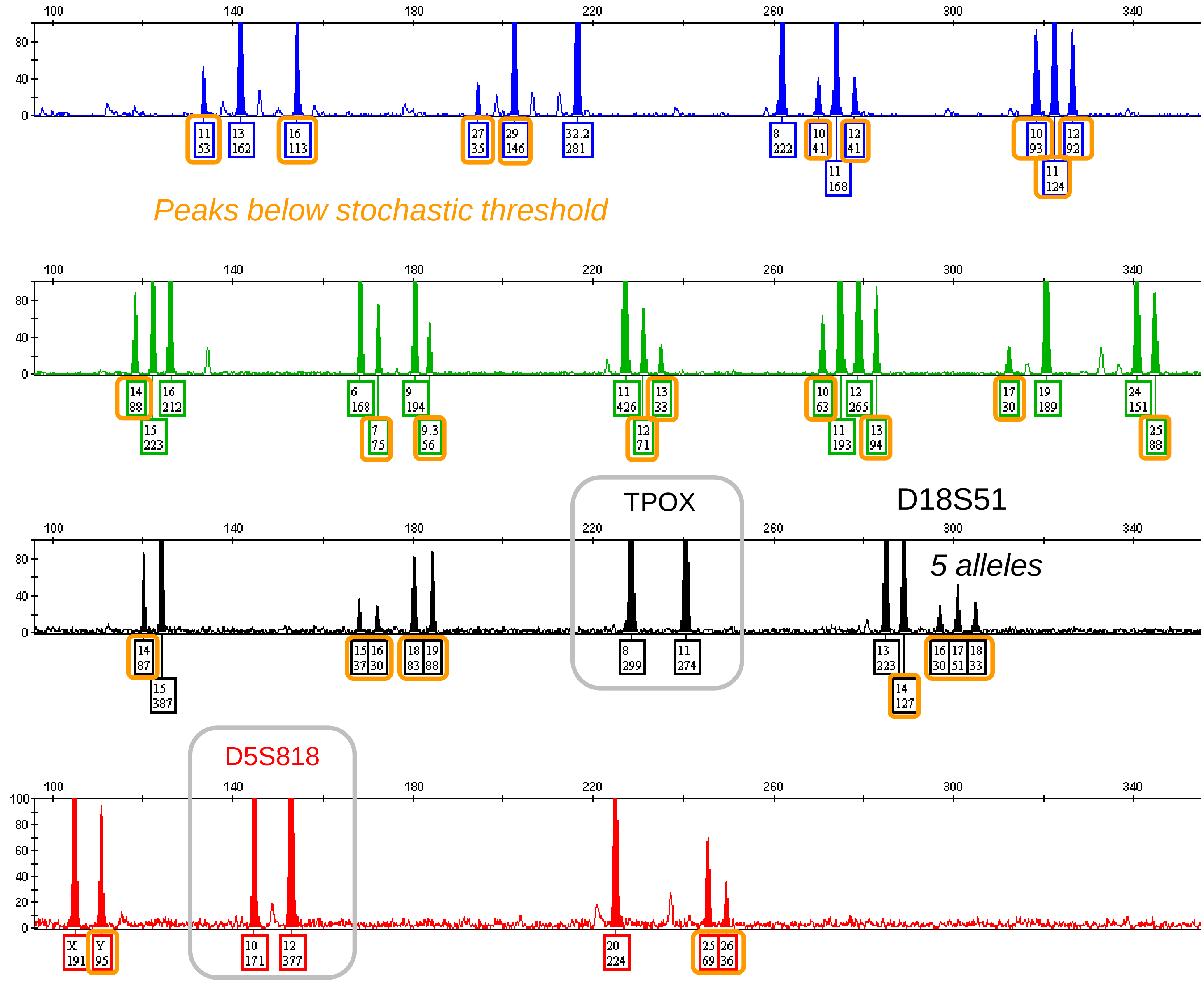
Identifiler

125 pg total DNA

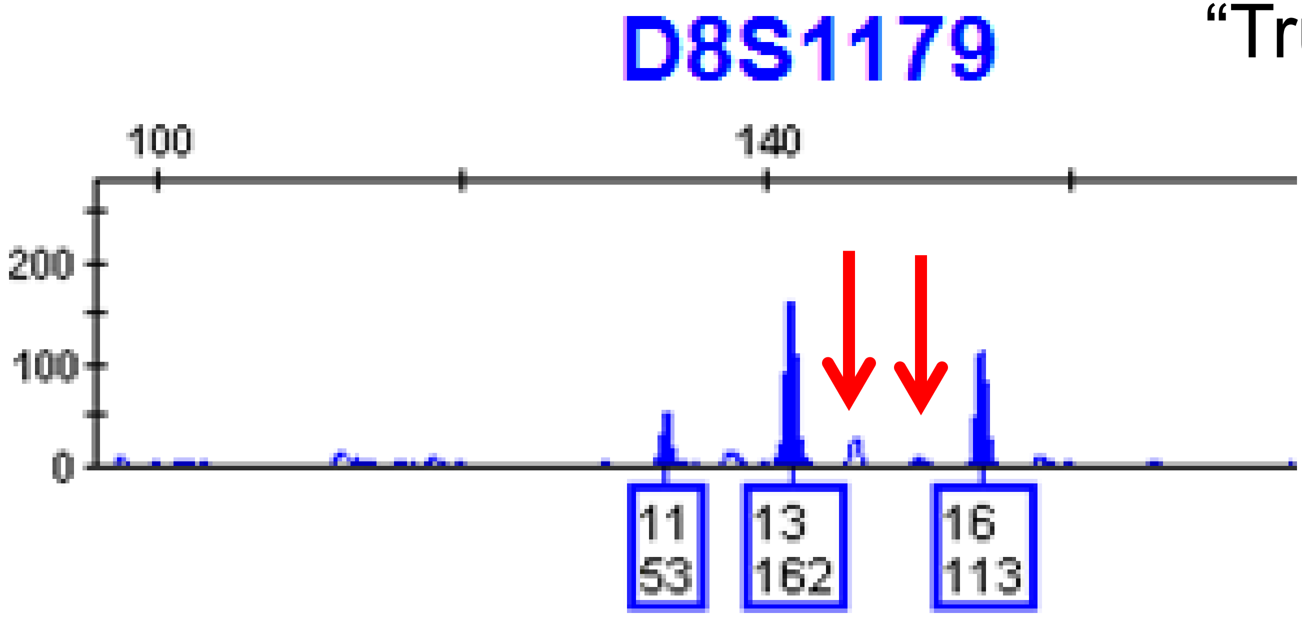
AT = 30 RFU

ST = 150 RFU

Stutter filter off



y-axis zoom to 100 RFU



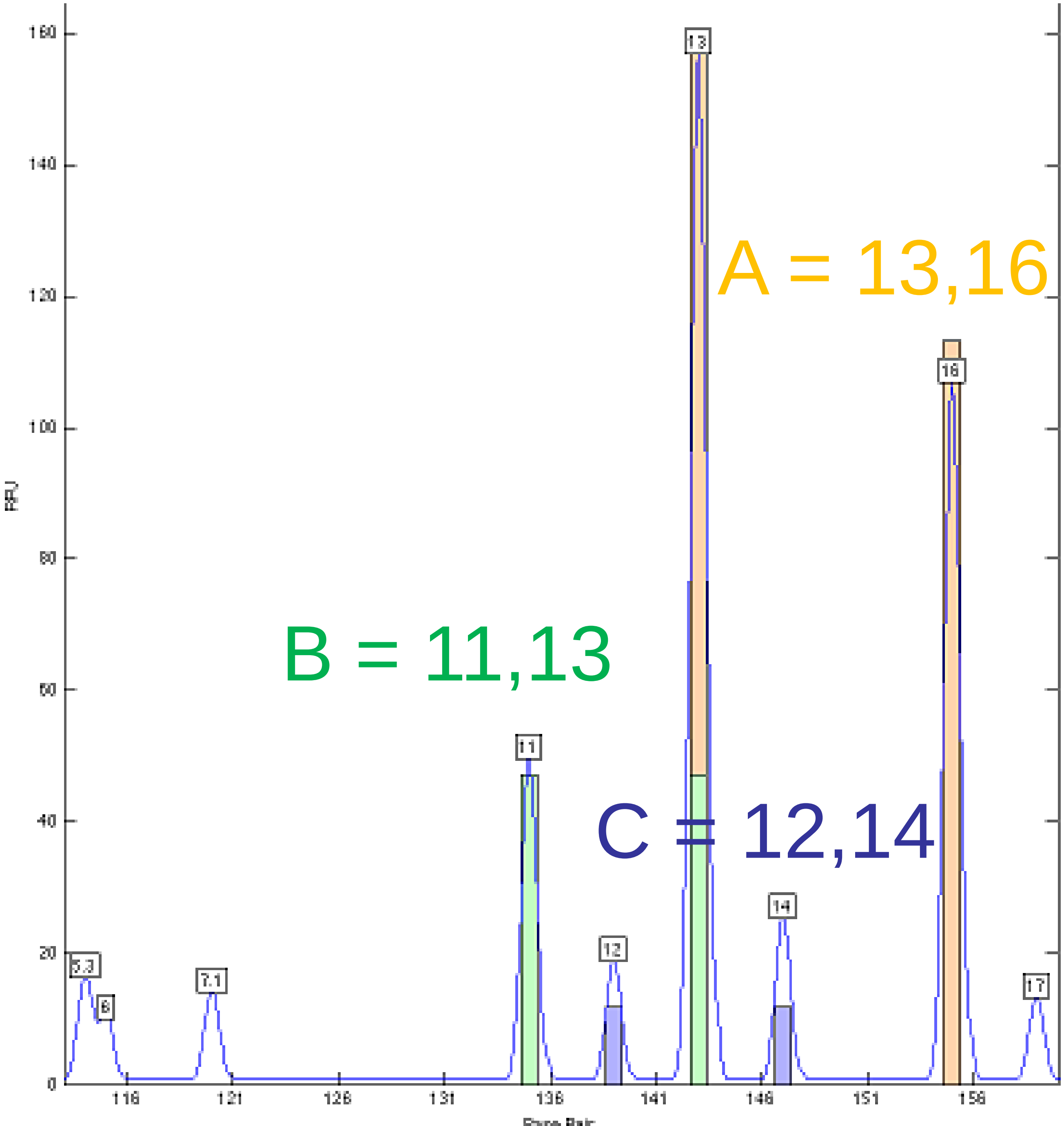
"True Genotypes"

A = 13, 16

B = 11, 13

C = 14, 15

3 person Mixture – No Conditioning
Major Contributor ≈ 83 pg input DNA
2 Minor Contributors ≈ 21 pg input DNA

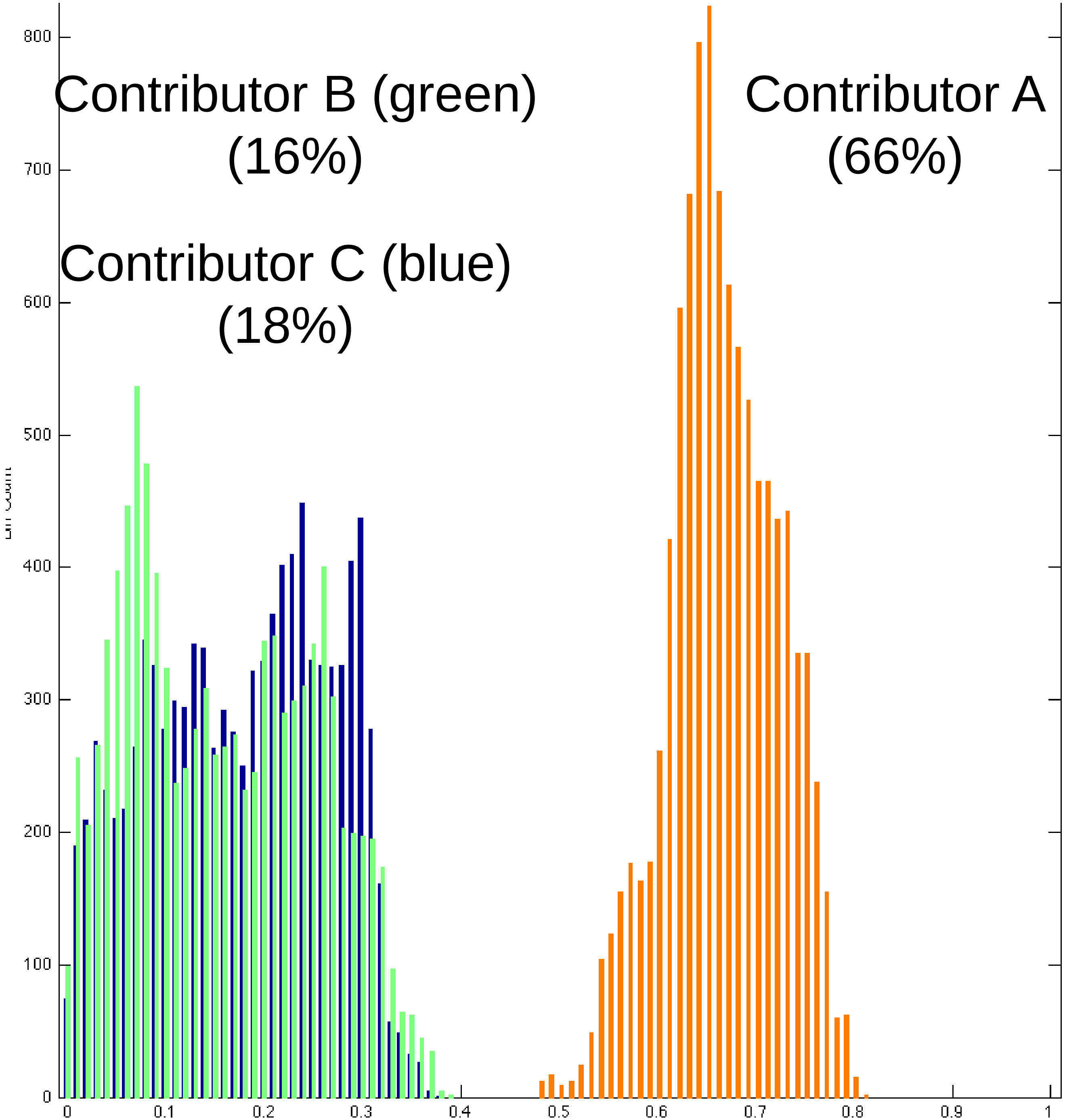


"True Genotypes"

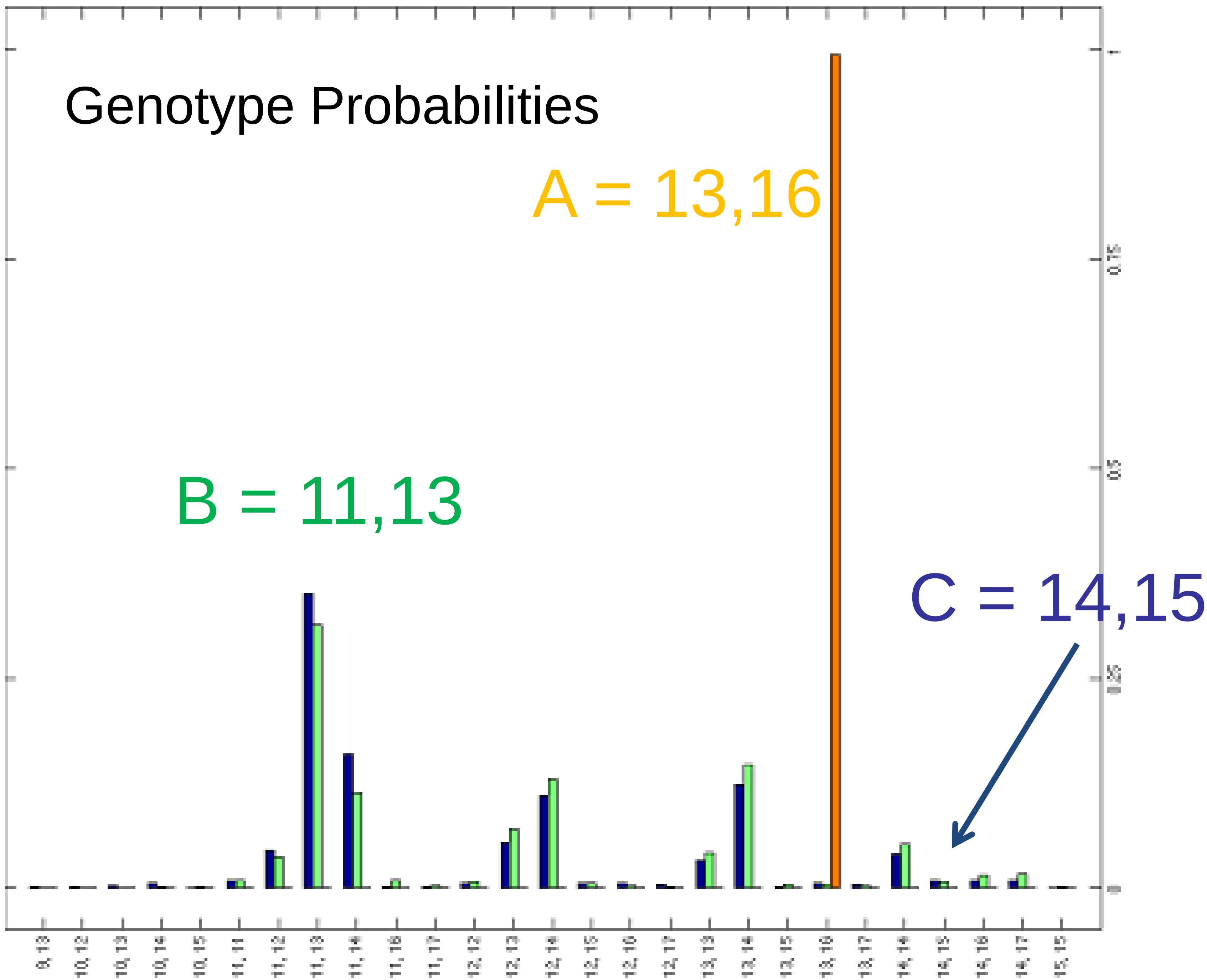
A = 13,16

B = 11,13

C = 14,15



Contributor B (green)
(16%)
Contributor C (blue)
(18%)
Contributor A
(66%)
800
700
600
500
400
300
200
100
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


Genotype Probabilities
A = 13,16
B = 11,13
C = 14,15

Results for Contributor A (male)

		Probability	Genotype		H_p	H_d	
Locus	**Allele Pair**	**Likelihood**	**Frequency**	**Suspect**	**Numerator**	**Denominator**	**LR**
CSF1PO	10, 11	0.572	0.1292			0.07395	
	11, 12	0.306	0.2133	1	0.30563	0.0652	
	10, 12	0.12	0.1547			0.01861	
					0.30563	0.15791	1.935
D13S317	11, 11	1	0.1149	1	1	0.11488	8.704
D8S1179	13, 16	0.998	0.0199	1	0.99786	0.0199	49.668

The match rarity between the evidence and suspect is 1.21 quintillion

Results for Contributor B (female)

		Probability	Genotype		H_p	H_d	
Locus	Allele Pair	Likelihood	Frequency	Suspect	Numerator	Denominator	LR
D8S1179	11, 13	0.073	0.0498	1	0.07338	0.00366	
	11, 14	0.034	0.0271			0.00092	
	13, 14	0.006	0.0996			0.00065	
	12, 14	0.011	0.0606			0.00068	
	12, 13	0.005	0.1115			0.0006	
	11, 12	0.018	0.0303			0.00054	
	14, 14	0.004	0.0271			0.00012	
	13, 13	0.003	0.0916			0.00031	
	14, 16	0.003	0.0108			0.00003	
	14, 15	0.001	0.0379			0.00003	

etc… 9.197

The match rarity between the evidence and suspect is 1.43 million

Results for Contributor C (male)

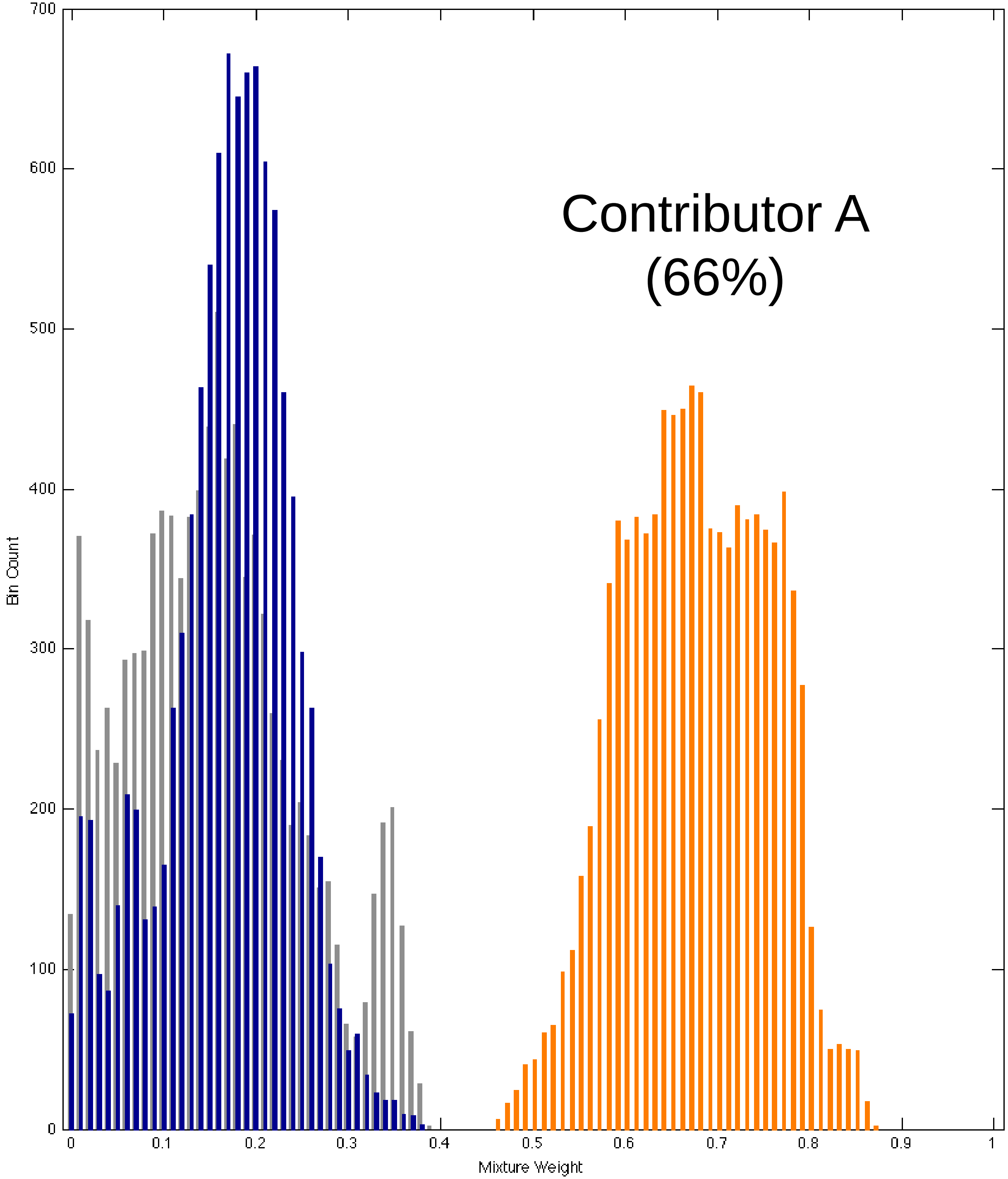
		Probability	Genotype		H_p	H_d	
Locus	**Allele Pair**	**Likelihood**	**Frequency**	**Suspect**	**Numerator**	**Denominator**	**LR**
D8S1179	11, 13	0.056	0.0498			0.00279	
	13, 14	0.007	0.0996			0.00066	
	12, 14	0.011	0.0606			0.00068	
	11, 14	0.021	0.0271			0.00056	
	12, 13	0.006	0.1115			0.00066	
	14, 14	0.005	0.0271			0.00013	
	etc…	etc…	etc…			etc…	
	14, 15	0.001	0.0379	1	0.00056	0.00002	
	12, 15	0.001	0.0424			0.00003	
	etc…	etc…	etc…			etc…	
	10, 15	0	0.0227			0.00001	
					0.00056	0.00665	**0.084**

The match rarity between the evidence and suspect is 9.16 thousand

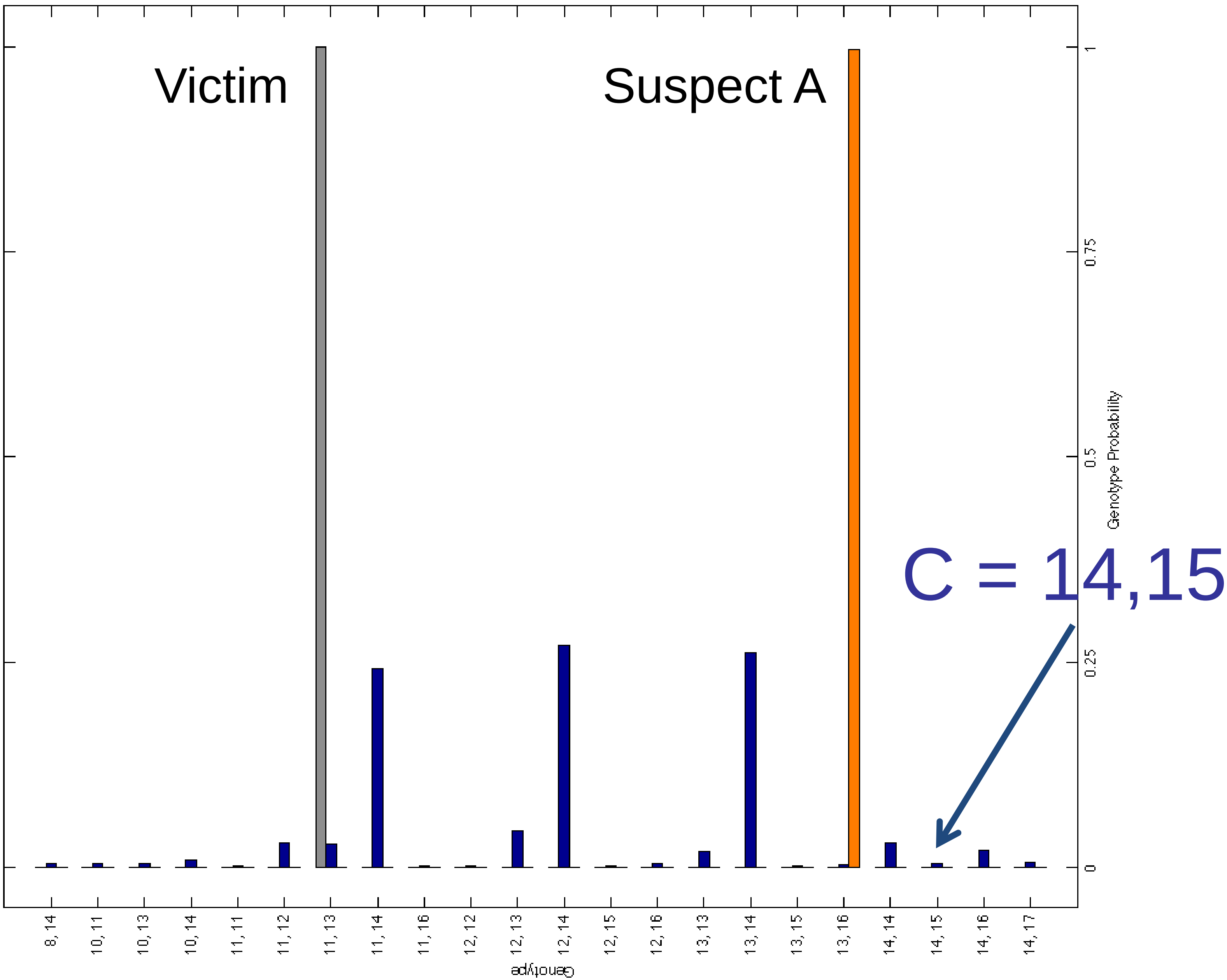
Contributor B (gray)
(16%)

Contributor C (blue)
(18%)

Conditioned on the Victim



The Power of Conditioning



The Power of Conditioning

	LR (no conditioning, 3unk)
Contributor A	**1.21 Quintillion**
Contributor B (victim)	**1.43 Million**
Contributor C	**9.16 Thousand**

	LR (conditioned on victim + 2unk)
Contributor A	**1.32 Quintillion**
Contributor B (victim)	**2.19 Million**
Contributor C	**59.8 Thousand**

Ranged from 1.13 to 800K

Summary

- True Allele utilizes probabilistic genotyping and makes better use of the data than the RMNE approach.

- However, the software is computer intensive. On our 4 processor system, it can take 12-16 hours to run up to four 3-person mixture samples.

Summary

- **Allele Sharing:** Stacking of alleles due to sharing creates more uncertainty.

- **Mixture Ratio:** With "distance" between the two contributors, there is greater certainty. Generally, True Allele performs better than RMNE and the classic LR with low level contributors.

Summary

- **DNA Quantity:** Generally, with high DNA signal, replicates runs on True Allele are very reproducible.
- However, with low DNA signal, higher levels of uncertainty are observed (as expected).
- There is a need to determine an appropriate threshold for an inclusion log(LR).

Thank you for your attention

Acknowledgments: NIJ & FBI Funding
Many slides from Mike Coble (NIST)

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Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm